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THE RELATION OF NUTRIENTS TO TOXICITY OF ARSENIC, BORAX, AND CHLORATE IN SOILS [1]

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INTRODUCTION

Much of the knowledge of chemical herbicides is empirical. Field practices in weed control are not adequately related to basic principles (*5*) [3] and consequently are unreliable or erratic. Proper interpretation of the abundant observational data on hand awaits the development of fundamental knowledge broad enough to encompass the wide range of variables in the field environment.

Essential to this knowledge is an understanding of the relative importance of the several edaphic factors most directly involved in toxicity relations. With herbicides that act through the soil, certainly the textural grade, water-holding capacity, and crop-producing power (fertility) are of paramount importance.

Three recent papers (*6*, *9*, *14*) explain the relation of textural grade and colloid content of soils to arsenic fixation and toxicity [4] and the effect of fertility upon chlorate toxicity. Briefly, heavy soils fix large amounts of arsenic, rendering it unavailable to plants. Arsenic toxicity is high in light soils, especially in those low in colloids. The reddish color of certain soils indicates the presence of compounds high in fixing power and capable of rendering arsenic unavailable (*9*, *14*). Chlorate toxicity in soils is inversely related to fertility, being high in infertile soils and low in those that produce heavy crops (*6*).

The extreme expression of these responses selected from a group of 80 California soils is illustrated in figure 1. Here are shown a chlorate (*A*) and an arsenic (*B*) toxicity series in Aiken clay loam. This soil is low in fertility and gives a definite response to application of 5 p. p. m. of sodium chlorate. On the other hand, it is fairly heavy, high in colloids, and red in color, and consequently arsenic toxicity is low. Arsenic toxicity, as related to colloid content and certain iron

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[3] Italic numbers in parentheses refer to Literature Cited, p. 671.

[4] The term "toxicity" has acquired a wide variety of meanings. For the present paper the criterion adopted is the application of chemical causing an almost complete suppression of growth. This use of the word has developed in the practice of soil sterilization because the practical object is to eliminate plants. In the interpretation of toxicity curves such as those of figures 3, 5, 7, 8, 9, 10, 11, and 12, the manner in which the individual graphs approach the base line is the basis used for comparison. Though the relations in the intermediate range may have theoretic interest, only the concept of toxicity that approaches in meaning the term "minimum lethal dose" has practical significance in weed control.

compounds, depends upon soil composition and cannot be readily altered. Fertility is the resultant of a number of factors, and chlorate toxicity might be considerably influenced if the particular ones concerned were known and understood.

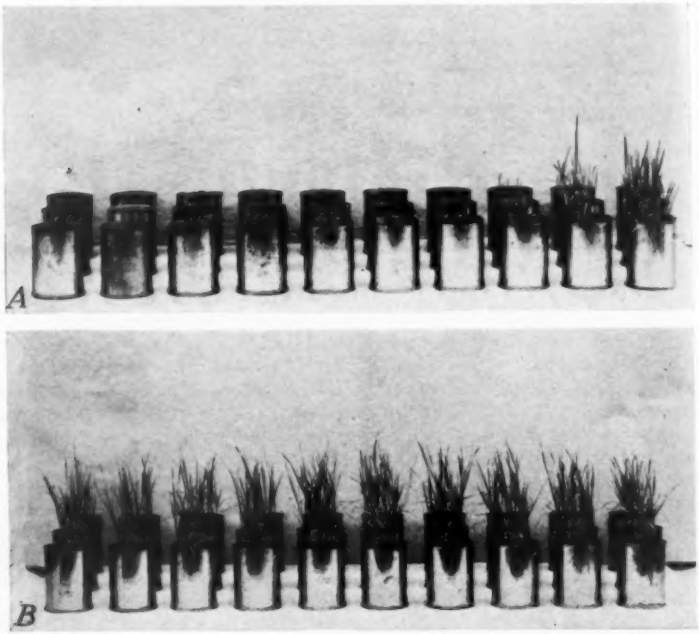

FIGURE 1.—Toxicity of sodium chlorate (*A*) and sodium arsenite (*B*) in Aiken clay loam, as shown by growth of Kanota oats. Checks are on the right, with concentrations increasing toward the left. The concentrations expressed in terms of $NaClO_3$ and As_2O_3 in air-dry soil are, from right to left, 0, 5, 15, 40, 80, 140, 220, 340, 490, and 680 p. p. m.

METHODS

Study of the plants growing in the cultures used in the experiments that have been described (*6*, *9*, *14*) indicated that those in infertile soils suffered definite mineral deficiencies. Since, in the chlorate experiments, there was a correlation between fertility and toxicity, similar greenhouse studies were begun with arsenic, borax, and chlorate in soils and in water cultures having four different levels of mineral nutrition. The nutrient levels were obtained by moistening the soil with salt solutions containing the principal nutrient elements in the proportions used by Hoagland in his culture solutions (*11*, *12*).

Table 1 summarizes descriptive data for the soils used. All samples were taken within 4 inches of the surface and, wherever possible, from fence lines where recent cultural operations had not affected the physical or chemical status of the soil.

TABLE 1.—*Name, sampling location, and moisture capacity of soils used in herbicide experiments*

No.	Type name	Sampling location (California)	Moisture [1]	Weight used
			Cubic centimeters	*Grams*
1	Fresno sandy loam	Hughson	75	600
2	Yolo fine sandy loam	Davis	85	500
3	Columbia fine sandy loam	Broderick	90	500
4	Arbuckle clay loam	Arbuckle	100	500
5	Aiken clay loam	Camino	115	400
6	Yolo adobe clay	Tracy	145	500
7	Yolo clay loam	Davis	150	500
8	Sacramento clay	Clarksburg	160	500
9	Stockton adobe clay	Stockton	160	500

[1] Volume of water required to moisten 500 g of air-dry soil to field capacity.

The method used to test toxicities consists in growing indicator plants (Kanota oats) in can cultures containing graded concentrations of the toxicant in the soil or solution used as the culture medium. Figure 1 illustrates two of these series. The data recorded are the height and fresh weight of the plants 30 days after planting.

Three toxicants were used in these tests, viz, sodium chlorate, sodium borate, and sodium arsenite. Concentrations are expressed in terms of arsenic trioxide (As_2O_3), anhydrous borax ($Na_2B_4O_7$), and sodium chlorate ($NaClO_3$), respectively.

In the chlorate cultures with complete nutrients (Hoagland's solution) added (see table 2), the nutrients were applied to the different soils in equal amounts. Concentrations are expressed as parts per million of the air-dry soil weights. For this reason the concentrations calculated on the basis of the soil moisture vary inversely with the field capacities of the soils. In the arsenic and borax cultures the nutrient elements were added in equal concentrations in the moistening solutions. In the tables, concentrations of nutrients are expressed in multiples of those that occur in Hoagland's full nutrient culture solution, and the symbol H is used to imply the nutrient concentrations that occur in a "normal" or "full" Hoagland's solution.[5] Iron was not used in the soil cultures but was included in the water cultures. The toxicants were added in the moistening solutions in like amounts in all soils, based on the air-dry weight. The concentrations run in a series as in previous experiments.

The methods so far described apply to the cultures used to test the effects of adding complete nutrient mixtures to chlorate-containing soils (table 2), arsenic-containing soils (table 3), borax-containing soils (table 4), and water cultures at different nutrient levels containing arsenic, borax, or chlorate (table 5). To find the effects of various single-salt amendments upon chlorate-containing soils, the experiments recorded in table 6 were performed. Soil cultures with the usual range of chlorate concentrations were replicated in 10 different soils in the greenhouse. The various series on each soil received the following: (1) Tap water; (2) 2H solution; (3) nitrate, 30 millimols of KNO_3 per liter; (4) nitrate, 30 millimols of $NaNO_3$ per liter; (5) nitrate, 15 millimols of $Ca(NO_3)_2$ per liter; (6) potash, 12 millimols of KNO_3 per liter; (7) potash, 12 millimols of KCl per liter; (8)

[5] This solution contains per liter 5 cc M KNO_3, 5 cc M $Ca(NO_3)_2$, 2 cc M $MgSO_4$, and 1 cc M KH_2PO_4.

potash, 6 millimols of K_2SO_4 per liter; (9) phosphate, 2 millimols of KH_2PO_4 per liter; (10) phosphate, 2 millimols of NaH_2PO_4 per liter; (11) phosphate, 2 millimols of H_3PO_4 per liter; (12) ammonia, 30 millimols of NH_4NO_3 per liter; (13) ammonia, 12 millimols of NH_3NO_3 per liter; (14) ammonia, 30 millimols of NH_4Cl per liter; (15) ammonia, 15 millimols of $(NH_4)_2SO_4$ per liter.

Finally, parallel series of experiments were run in water cultures, with graded chlorate concentrations in various single-salt solutions (table 7). The plants were started in sand, transferred to $\frac{H}{10}$ solution, where they remained for 7 days to become established, and then shifted to the chlorate-containing culture solutions.

RESULTS OF EXPERIMENTS

TOXICITY IN SOILS TREATED WITH COMPLETE NUTRIENTS

Chlorate

Data on the toxicity of sodium chlorate at four nutrient levels in nine soils are presented in table 2. The values given are the average height and the total fresh weight for the 10 plants grown in each can. To better illustrate these results, figure 2 shows sets of plants in Yolo sandy loam, Aiken clay loam, and Yolo adobe clay. Extreme effects of plant nutrients in lowering chlorate toxicity are shown in the Aiken soil.

Table 2.—*Toxicity of sodium chlorate at 4 nutrient levels in 9 California soils as shown by growth of Kanota oats*

[Plants harvested Feb. 17, 1936]

AIKEN CLAY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2.2 H		4.3 H		8.7 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Gram*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	6	0.3	23	5.0	30	7.1	30	7.1
15	6	.2	25	5.6	20	4.8	28	6.9
40	3	.1	17	2.9	20	3.7	25	5.9
80	0	.0	14	2.1	18	3.4	23	4.6
140	0	.0	15	1.6	19	2.4	26	5.0
220	0	.0	8	.5	16	1.8	21	3.2
340	0	.0	5	.3	9	.7	22	2.8
490	0	.0	5	.2	5	.3	10	1.0
680	0	.0	3	.1	5	.1	8	.6
Check	8	.4	20	4.6	24	6.3	29	7.4

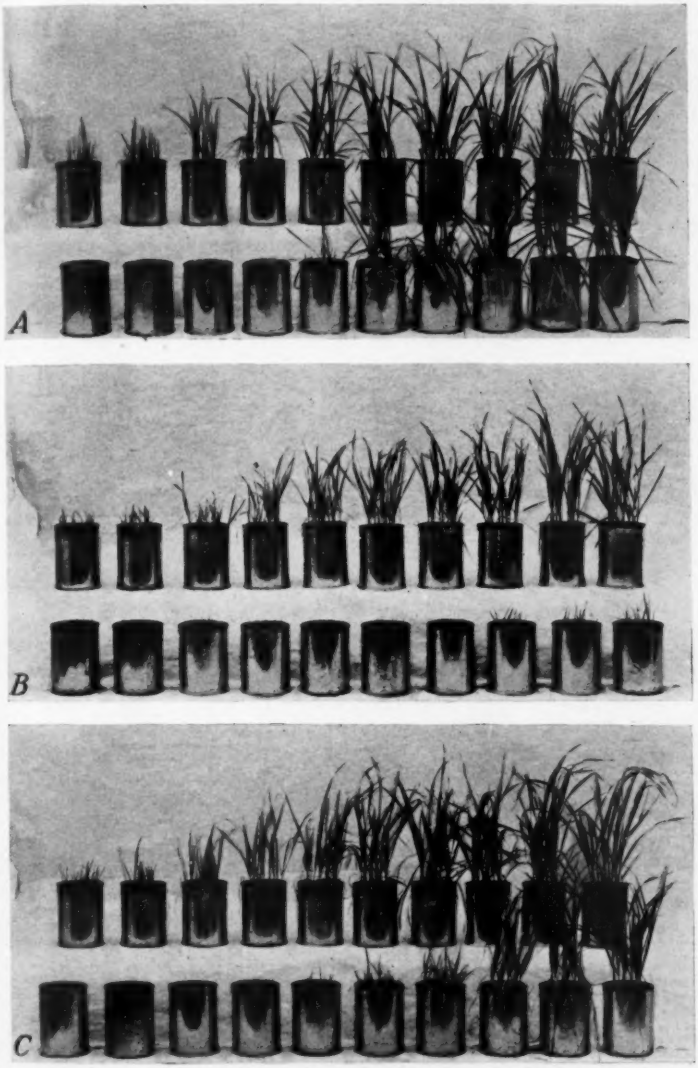

FIGURE 2.—Chlorate toxicity in three California soils in relation to nutrient level: *A*, Yolo sandy loam; *B*, Aiken clay loam; *C*, Yolo adobe clay. The lower series in each soil is the check; the checks were moistened with chlorate in tap water. The upper series received plant nutrients in the applied moistening solution approximately equivalent to a 6H solution in the Yolo sandy loam, a 4H solution in the Aiken clay loam, and a 3H solution in the Yolo adobe clay. The concentrations expressed in terms of $NaClO_3$ in air-dry soil, from right to left, are 0, 5, 15, 40, 80, 140, 220, 340, 490, and 680 p. p. m.

TABLE 2.—*Toxicity of sodium chlorate at 4 nutrient levels in 9 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested Feb. 17, 1936]

ARBUCKLE CLAY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2.5 H		5.0 H		10.0 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	24	4.8	33	10.4	36	13.9	38	11.1
15	29	6.6	32	10.5	36	13.0	37	10.9
40	16	.9	30	9.4	34	11.5	35	10.4
80	8	.5	28	7.1	33	9.8	33	9.4
140	5	.1	21	4.5	28	6.0	31	6.6
220	4	.1	16	1.2	22	3.3	27	5.0
340	3	.0	8	.6	15	1.2	23	3.2
490	0	.0	5	.3	8	.4	16	1.2
680	0	.0	5	.2	5	.2	13	.9
Check	25	4.4	33	10.9	34	14.3	40	13.2

STOCKTON ADOBE CLAY

$NaClO_3$ in air-dry soil (p. p. m.)	Water		1.6 H		3.1 H		6.2 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	25	5.0	31	9.8	34	12.1	39	13.8
15	25	4.0	33	10.1	35	11.1	37	14.1
40	17	1.4	32	7.8	35	10.8	39	14.2
80	7	.4	28	6.6	34	5.9	39	12.6
140	6	.1	23	5.9	28	4.7	36	9.9
220	4	.1	19	1.8	22	3.0	32	6.7
340	0	.0	10	.6	17	1.2	25	3.6
490	0	.0	8	.6	10	.6	24	2.9
680	0	.0	6	.3	9	.5	22	2.0
Check	25	5.5	30	9.5	32	12.5	40	14.9

YOLO ADOBE CLAY

$NaClO_3$ in air-dry soil (p. p. m.)	Water		1.7 H		3.4 H		6.9 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	28	5.8	34	10.9	37	13.1	35	12.2
15	22	3.0	31	8.9	33	12.3	37	12.5
40	15	1.2	31	8.1	31	10.4	34	9.5
80	9	.5	26	5.3	31	7.5	35	10.0
140	6	.2	24	4.0	26	5.2	33	7.5
220	4	.1	17	1.6	22	3.3	30	4.8
340	0	.0	8	.5	15	1.3	25	3.2
490	0	.0	7	.5	9	.6	17	1.4
680	0	.0	4	.3	7	.3	11	.8
Check	27	5.6	34	10.3	36	13.0	37	12.4

TABLE 2.—*Toxicity of sodium chlorate at 4 nutrient levels in 9 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested Feb. 17, 1936]

YOLO FINE SANDY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2.9 H		5.9 H		11.8 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	27	5.6	31	9.7	32	8.9	28	5.0
15	28	4.8	31	9.8	34	9.4	30	5.7
40	29	4.9	31	8.8	33	8.3	32	6.9
80	24	3.2	29	8.0	32	7.8	33	6.9
140	13	1.1	29	5.7	30	5.9	28	4.3
220	5	.1	24	3.5	24	4.0	25	3.4
340	4	.1	13	.9	19	1.7	20	2.4
490	0	.0	13	.5	12	1.0	16	1.5
680	0	.0	5	.2	11	.6	12	.8
Check	28	5.6	32	9.4	30	8.5	29	4.7

FRESNO SANDY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		3.3 H		6.7 H		13.3 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	32	8.4	35	10.7	32	8.3	27	4.0
15	31	8.3	34	9.0	31	6.0	26	4.0
40	30	6.0	34	8.6	32	6.0	25	3.4
80	23	3.3	31	6.9	28	5.4	22	2.9
140	18	1.8	28	5.0	26	4.3	22	2.6
220	8	.5	24	3.1	21	2.9	19	2.0
340	0	.0	19	1.9	17	1.5	13	1.0
490	0	.0	10	.7	8	.6	12	.8
680	0	.0	0	.0	4	.2	9	.4
Check	29	7.9	36	11.8	34	9.4	28	4.7

SACRAMENTO CLAY

$NaClO_3$ in air-dry soil (p. p. m.)	Water		1.5 H		3.1 H		6.2 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Gram*	*Centimeters*	*Gram*	*Centimeters*	*Gram*	*Centimeters*	*Gram*
5	27	9.0	32	9.5	30	8.9	31	8.7
15	27	8.4	30	9.1	30	8.3	32	8.5
40	26	7.3	28	8.0	28	7.5	31	7.9
80	25	6.3	26	7.3	28	7.2	29	7.9
140	20	4.4	26	6.1	28	7.0	28	7.3
220	14	2.1	24	4.8	26	6.0	28	6.0
340	7	.7	18	3.7	22	4.5	24	5.0
490	4	.4	12	2.6	18	3.0	22	3.6
680	3	.1	10	1.2	10	1.8	19	2.4
Check	26	8.6	31	10.1	35	9.5	33	8.9

TABLE 2.—*Toxicity of sodium chlorate at 4 nutrient levels in 9 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested Feb. 17, 1936]

COLUMBIA FINE SANDY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2.8 H		5.5 H		11.0 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	33	11.0	34	9.5	32	8.5	31	6.5
15	32	10.4	33	9.1	32	9.2	31	6.7
40	33	8.8	33	9.4	35	9.7	31	6.7
80	31	6.5	32	7.7	32	7.7	30	6.4
140	27	4.4	30	6.0	30	6.7	30	6.5
220	25	4.1	28	5.4	25	4.5	29	5.8
340	13	1.0	20	2.5	23	3.2	25	3.4
490	9	.6	19	2.1	19	2.0	21	2.3
680	5	.1	14	1.0	16	1.5	18	1.7
Check	34	11.4	33	9.8	32	9.3	30	7.1

YOLO CLAY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		1.7 H		3.3 H		6.7 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	33	17.4	37	16.0	39	15.7	43	13.0
15	36	16.5	38	16.2	39	14.8	40	12.1
40	31	11.3	39	15.8	40	14.6	41	14.4
80	31	9.1	37	13.8	38	13.5	41	14.4
140	29	4.9	34	9.4	33	10.4	40	11.8
220	20	2.6	31	7.0	30	7.8	35	9.0
340	9	.9	25	3.3	27	3.8	30	5.5
490	6	.3	15	2.0	22	2.9	30	5.3
680	4	.1	8	.9	16	1.3	24	2.1
Check	35	17.0	38	17.5	41	15.9	38	13.5

Data from table 2 showing the relation of nutrient level to chlorate toxicity in Yolo fine sandy loam, Yolo adobe clay, and Aiken clay loam are plotted in figure 3. In Yolo adobe clay increasing the nutrient supply reduced the toxicity until at the highest level the plants survived at a chlorate concentration of 680 p. p. m. In the Aiken soil the results were similar. In the Yolo fine sandy loam the extremely high nutrient concentration at the higher levels caused some reduction in growth. Judging from the appearance of the plants, this resulted from excessive nitrates. However, at chlorate concentrations of 340 p. p. m. and over, the curves have the same relative positions as they do in the other soils. This same relation appeared with other soils tested, indicating that the higher the nutrient level in the soil the lower the chlorate toxicity, even where the nutrients are so concentrated as to reduce growth in the chlorate-free check cultures.

ARSENIC

Similar tests were carried out with arsenic and borax on seven of the same soils. To facilitate the setting up of the experiments and to aid in comparisons and analyses of the data, nutrients were added in like concentrations in the moistening solutions. Data on the arsenic tests appear in table 3, and results are illustrated in figures 4 and 5.

Although the soils and crops varied widely in these tests, convergence of the curves for all nutrient levels at the base line confirm the conclusion that arsenic toxicity is unrelated to the soil's fertility but is determined by its textural grade.

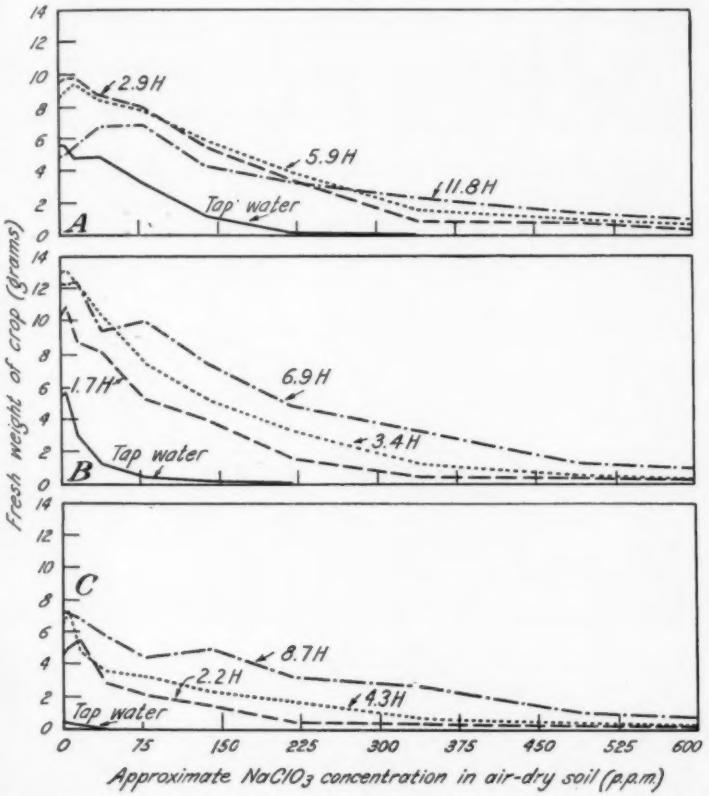

FIGURE 3.—Relation of chlorate toxicity to nutrient level in the soil: *A*, Yolo fine sandy loam; *B*, Yolo adobe clay; *C*, Aiken clay loam. The approximate nutrient level of each culture series is indicated on the individual curves, expressed, in terms of the soil solution, as multiples of concentrations used in a standard Hoagland's nutrient solution.

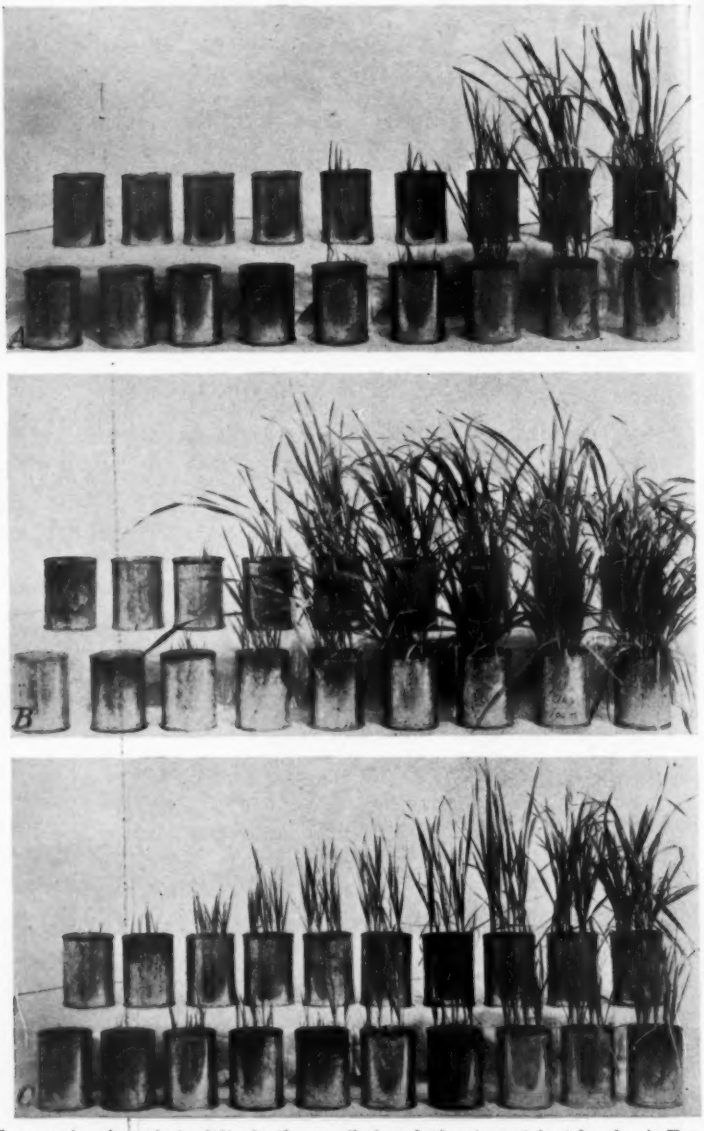

FIGURE 4.—Arsenic toxicity in three soils in relation to nutrient level: *A*, Fresno sandy loam; *B*, Yolo clay loam; *C*, Stockton adobe clay. The lower series in each soil has been moistened with sodium arsenite in tap water; the upper contains like arsenic concentrations applied in a 4H solution. The concentrations, expressed in terms of As_2O_3 in air-dry soil from right to left, are 0, 15, 40, 80, 140, 220, 340, 490, and 680 p. p. m. A 920 p. p. m. culture is included in the Stockton adobe clay.

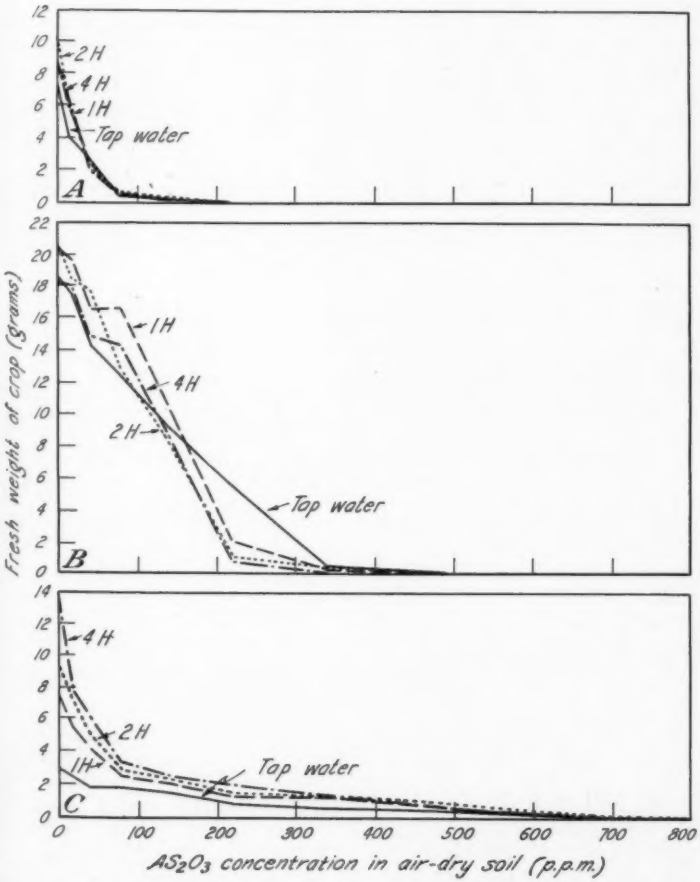

FIGURE 5.—Arsenic toxicity at four nutrient levels in three soils: *A*, Fresno sandy loam; *B*, Yolo clay loam; *C*, Stockton adobe clay.

TABLE 3.—*Toxicity of sodium arsenite at 4 nutrient levels in 7 California soils as shown by growth of Kanota oats*

[Plants harvested Apr. 24, 1937]

As_2O_3 in air-dry soil (p. p. m.)	Water		1 H		2 H		4 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
FRESNO SANDY LOAM								
15	26	4.0	31	5.8	32	5.7	30	6.3
40	24	2.6	22	2.3	22	2.0	17	1.9
80	8	.5	8	.4	9	.6	8	.4
140	7	.2	6	.2	6	.3	6	.2
220	0	.0	0	.0	0	.0	0	.0
340	0	.0	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	27	7.2	32	8.8	35	10.3	35	8.8
COLUMBIA FINE SANDY LOAM								
15	29	11.1	34	10.5	31	11.1	33	8.9
40	32	9.9	32	9.3	30	8.4	27	7.0
80	27	5.5	28	5.8	26	5.0	30	4.7
140	11	1.4	17	1.9	15	1.3	9	.8
220	6	.2	5	.1	6	.3	0	.0
340	0	.0	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	30	13.5	33	13.3	31	12.3	31	10.6
YOLO FINE SANDY LOAM								
15	25	5.5	27	7.5	26	7.9	30	8.3
40	23	4.7	25	6.1	27	6.8	29	6.8
80	23	4.4	26	6.2	26	5.1	28	5.6
140	17	2.4	23	3.3	25	3.7	15	1.4
220	7	.4	9	.6	7	.3	8	.5
340	0	.0	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	25	5.4	26	7.4	28	8.8	33	8.9
YOLO CLAY LOAM								
15	31	17.5	33	19.6	34	18.5	36	18.0
40	28	14.2	34	16.5	37	17.8	38	14.9
80	30	12.4	35	16.6	31	12.8	33	14.2
140	33	8.9	34	10.5	20	8.1	33	8.7
220	26	5.4	10	2.0	12	1.0	7	.6
340	6	.3	6	.2	5	.3	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	33	18.6	33	20.2	34	20.4	35	18.3
ARBUCKLE CLAY LOAM								
15	24	4.2	26	7.2	30	7.8	32	10.5
40	23	3.6	26	5.8	30	7.5	31	7.1
80	19	3.3	27	5.5	29	6.0	31	6.0
140	22	3.0	25	4.2	30	4.6	31	4.9
220	18	2.9	14	1.8	15	2.0	18	1.9
340	10	1.5	18	1.5	11	1.2	12	.9
490	7	.4	8	.4	7	.3	10	.6
680	0	.0	0	.0	6	.1	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	25	4.5	27	7.4	28	8.4	33	12.1

TABLE 3.—*Toxicity of sodium arsenite at 4 nutrient levels in 7 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested Apr. 24, 1937]

STOCKTON ADOBE CLAY

As_2O_3 in air-dry soil (p. p. m.)	Water		1 H		2 H		4 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
15	16	2.3	24	5.5	26	7.2	26	7.3
40	16	1.8	22	4.0	22	4.8	30	6.9
80	17	1.8	20	2.5	23	2.9	27	3.2
140	16	1.5	17	2.0	18	2.3	20	2.4
220	11	.8	13	1.3	13	1.5	17	2.0
340	8	.6	11	1.3	13	1.4	13	1.4
490	7	.4	9	.7	10	1.0	9	.7
680	5	.1	5	.1	5	.2	6	.2
920	0	.0	0	.0	0	.0	0	.0
Check	17	2.9	24	7.4	24	9.2	33	14.1
AIKEN CLAY LOAM								
15	23	3.5	26	4.7	25	4.8	29	5.8
40	23	3.4	26	4.0	24	4.3	26	4.7
80	21	2.9	23	3.9	27	4.2	26	4.2
140	21	2.4	23	3.7	23	3.7	24	3.6
220	21	2.2	19	3.0	22	3.0	21	2.9
340	22	2.1	19	2.4	18	2.3	20	2.5
490	17	1.8	17	2.2	16	1.8	18	2.2
680	10	1.1	14	1.8	15	1.3	11	1.2
920	8	.7	14	1.2	12	1.2	7	.7
Check	22	3.2	25	5.1	23	5.3	27	5.9

BORAX

Boron compounds, being nonpoisonous to animals, offer promise as soil sterilizers (*7*, *8*). Tests on a number of different boron salts indicate that the content of elemental boron, and not the form of compound in which it occurs, is the major factor involved in toxicity. Herbicidal use depends on solubility as well as toxicity (*8*). Borax, being readily soluble, was used in the toxicity experiments. Concentrations are expressed in terms of anhydrous borax ($Na_2B_4O_7$).

Table 4 contains the data on borax toxicity at four nutrient levels in seven soils. Results in Frenso sandy loam, Arbuckle clay loam, and Stockton adobe clay are shown in figures 6 and 7. As with arsenic, the toxicity evidently varies with textural grade, being lower in the heavy soils. Expressed as crop reduction compared with the check yield, toxicity seems unrelated to fertility. Although borax is held in soils much less firmly than arsenic against the leaching effects of moving water (*8*), the results illustrated here show that it behaves much like arsenic in its toxicity relations.

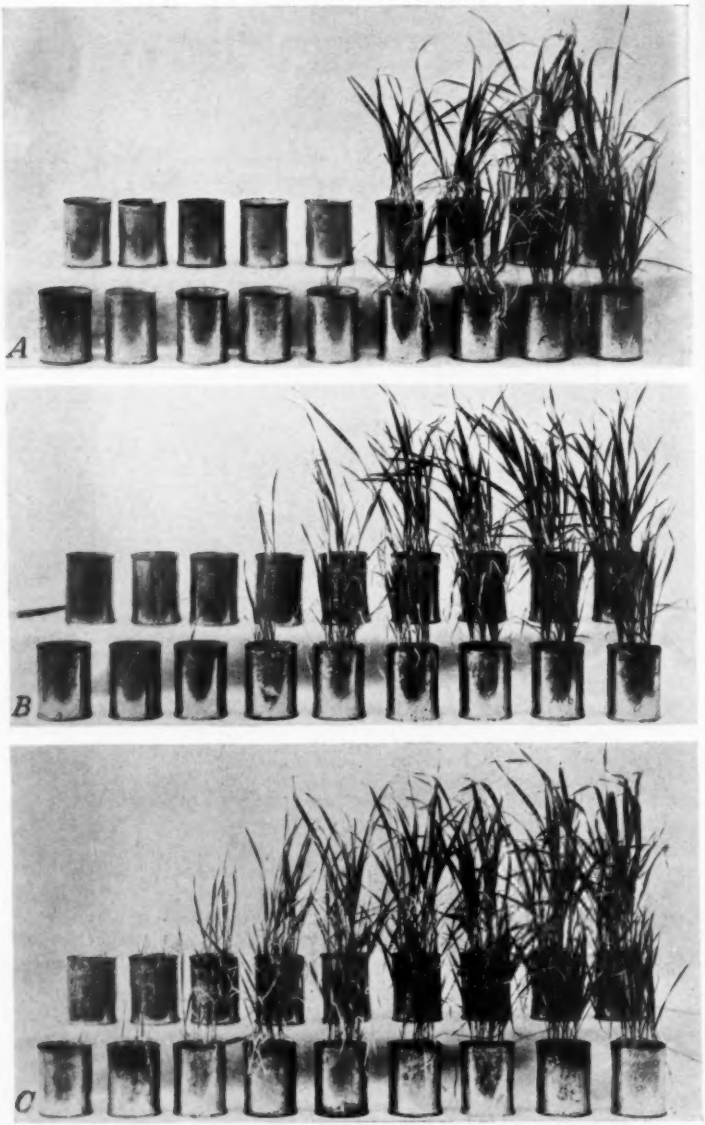

FIGURE 6.—Borax toxicity in three soils in relation to nutrient level: *A*, Fresno sandy loam; *B*, Arbuckle clay loam; *C*, Stockton adobe clay. The top row of cultures in each soil was moistened with 4H solution, the lower with tap water. The concentrations, expressed in terms of $Na_2B_4O_7$ in air-dry soil, from right to left, are 0, 15, 40, 80, 140, 220, 340, 490, and 680 p. p. m.

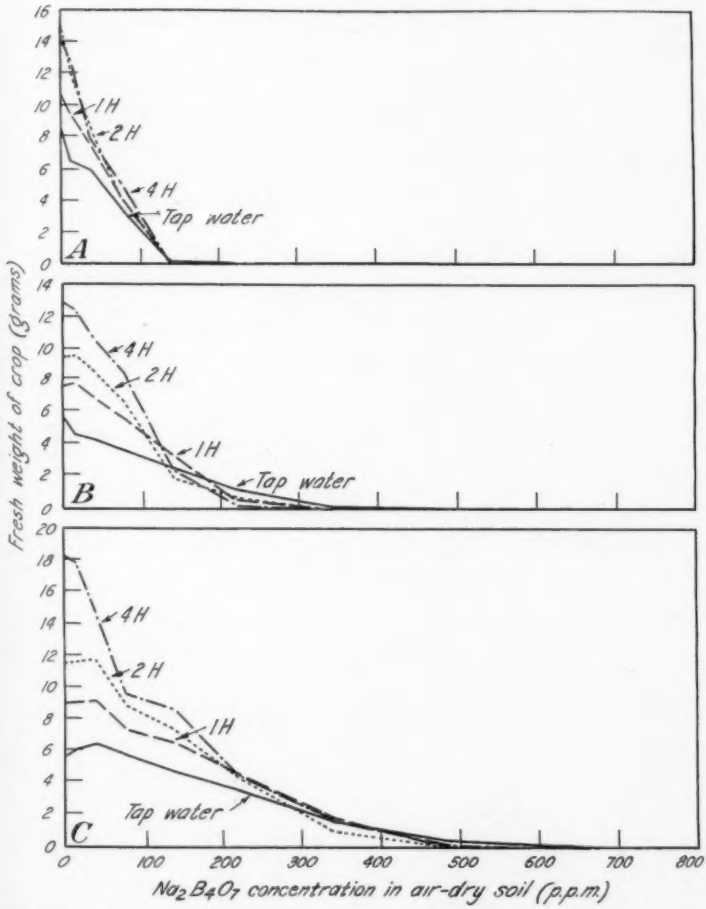

FIGURE 7.—Borax toxicity at four nutrient levels in three soils: *A*, Fresno sandy loam; *B*, Arbuckle clay loam; *C*, Stockton adobe clay.

TABLE 4.—*Toxicity of boraz at 4 nutrient levels in 7 California soils as shown by growth of Kanota oats*

[Plants harvested Apr. 22, 1936]

$Na_2B_4O_7$ in air-dry soil (p. p. m.)	Water		1 H		2 H		4 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
FRESNO SANDY LOAM								
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
15	34	6.5	37	9.2	42	11.6	45	12.5
40	35	5.7	37	7.4	40	8.0	37	7.2
80	23	2.6	30	3.4	33	3.2	35	3.7
140	6	.2	0	.0	0	.0	0	.0
220	0	.0	0	.0	0	.0	0	.0
340	0	.0	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	34	8.5	37	10.7	42	15.0	45	14.2
YOLO FINE SANDY LOAM								
15	27	4.9	30	8.6	33	9.8	37	7.2
40	27	4.7	31	7.2	33	8.8	34	8.8
80	27	3.0	26	2.7	27	3.5	30	4.8
140	8	.5	8	.4	7	.2	7	.2
220	0	.0	0	.0	5	.1	0	.0
340	0	.0	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	27	6.7	32	9.9	33	11.2	36	9.3
COLUMBIA FINE SANDY LOAM								
15	40	13.4	40	14.3	40	16.3	45	17.1
40	39	12.8	43	13.8	43	13.6	46	12.0
80	34	7.5	36	8.7	39	11.5	41	11.1
140	18	1.2	12	1.5	20	1.8	11	1.0
220	0	.0	0	.0	0	.0	0	.0
340	0	.0	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	40	15.0	43	16.7	45	17.7	46	16.7
ARBUCKLE CLAY LOAM								
15	27	4.5	32	7.7	32	9.5	37	12.2
40	27	4.1	31	6.8	33	8.4	36	10.4
80	27	3.4	29	5.5	32	6.5	37	8.2
140	26	2.4	27	3.2	20	1.9	26	2.3
220	15	1.1	10	.8	10	.8	5	.2
340	7	.2	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	27	5.6	30	7.5	32	9.3	35	12.8
YOLO CLAY LOAM								
15	39	18.0	38	18.7	40	19.0	42	19.6
40	38	16.4	42	14.8	42	17.2	41	16.2
80	37	13.7	40	13.8	40	14.9	43	14.9
140	37	10.1	38	10.0	37	8.1	36	7.8
220	18	1.2	8	.7	24	1.7	8	1.5
340	0	.0	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	37	19.5	37	20.7	40	21.2	40	22.1

TABLE 4.—*Toxicity of borax at 4 nutrient levels in 7 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested Apr. 22, 1936]

AIKEN CLAY LOAM

$Na_2B_4O_7$ in air-dry soil (p. p. m.)	Water		1 H		2 H		4 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
15	24	2.8	24	3.3	26	3.9	30	5.2
40	24	2.5	24	3.3	26	3.9	30	5.0
80	22	2.2	24	3.4	25	3.6	28	4.5
140	20	1.9	23	3.0	24	2.9	24	3.4
220	19	1.7	19	2.4	18	2.2	19	2.1
340	10	1.0	10	1.3	10	1.1	9	.8
490	3	.3	5	.4	5	.3	5	.2
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	22	2.7	24	3.3	26	3.8	31	5.6
STOCKTON ADOBE CLAY								
15	31	6.0	33	9.0	38	11.5	42	17.8
40	30	6.4	36	9.1	39	11.7	41	14.7
80	31	5.7	34	7.2	36	8.9	37	10.5
140	31	4.7	33	6.6	39	7.4	37	8.7
220	27	3.7	30	4.7	32	4.4	33	4.5
340	19	1.5	21	1.8	17	1.0	12	1.6
490	7	.5	0	.0	0	.0	6	.2
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	29	5.5	34	9.0	37	11.4	42	18.1

TOXICITY IN WATER CULTURES WITH COMPLETE NUTRIENTS

To find the effects of the soils upon the action of the chemicals under investigation, and to set a standard for comparing the various soils and chemicals, water-culture experiments were conducted at four nutrient levels and with varying concentrations of the toxicants. The results with three chemicals are reported in table 5, and these data are plotted in figure 8.

The outstanding point in these data is the extreme toxicity of arsenic. This chemical was less toxic than chlorate in some soils (for instance, Aiken clay loam, as shown in fig. 1). The soil profoundly affects arsenic toxicity (*14*), reducing it manyfold even in the coarser soils, where arsenic is highly toxic. Since arsenic toxicity is not affected by nutrient level, the solubility or availability to the plant seems ch efly

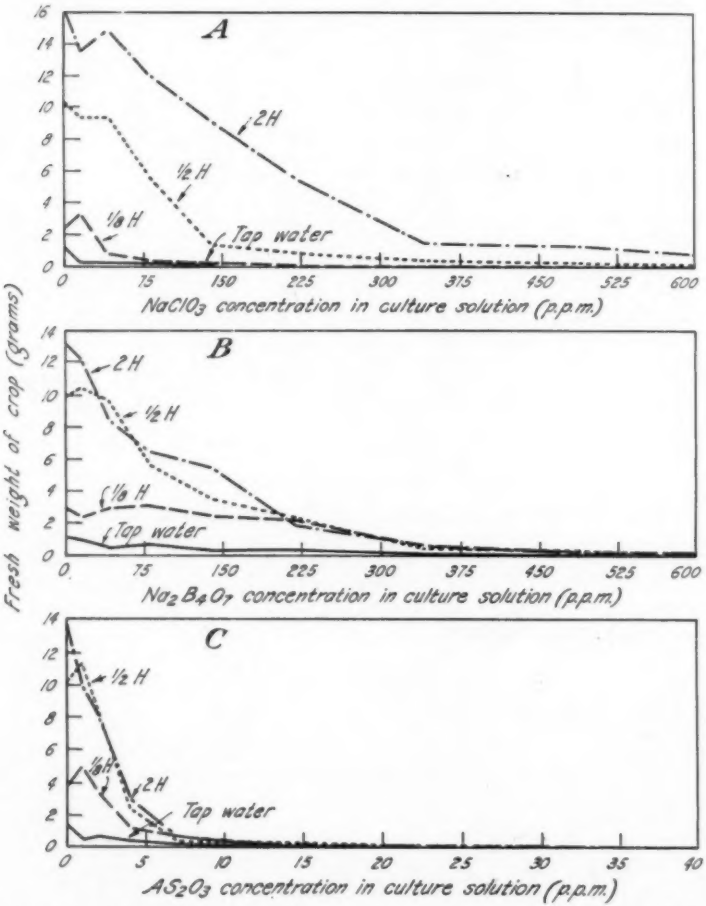

FIGURE 8.—Chlorate (*A*), borax (*B*), and arsenic (*C*) toxicity in culture solutions at four nutrient levels. Note the extremely high toxicity of arsenic, the scale being 15 times that of the other graphs.

concerned. To provide a lethal concentration in the soil, from 10 to 100 times the amount needed in the soil solution must be applied, depending upon the textural grade of the soil. The relation of fixation by the soil to arsenic toxicity has been discussed in a current publication (*14*).

TABLE 5.—*Toxicity of sodium chlorate, sodium arsenite, and borax in water cultures at 4 nutrient levels as shown by growth of Kanota oats*

[Plants harvested June 12, 1936]

SODIUM CHLORATE

Toxicant in culture solution (p. p. m.)	Water		¼ H		½ H		2 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
15	8	0.2	17	3.3	20	9.4	37	13.7
40	7	.2	8	.9	27	9.4	38	14.9
80	7	.2	8	.5	25	5.8	38	12.1
140	6	.1	9	.5	15	1.5	34	9.1
220	6	.1	8	.3	13	.9	25	5.6
340	6	.1	7	.2	10	.4	12	1.6
490	6	.1	7	.1	9	.3	12	1.4
680	5	.1	6	.1	8	.3	10	.6
920	5	.1	6	.1	7	.2	7	.4
Check	9	1.3	15	2.5	30	10.3	45	16.1
SODIUM ARSENITE								
1	8	0.5	21	5.0	31	11.5	39	9.9
2	8	.8	18	3.6	25	8.5	35	8.1
4	7	.4	10	1.2	20	2.4	26	3.1
7	7	.2	10	.8	18	.5	12	.8
11	6	.2	7	.4	9	.5	8	.4
16	6	.1	6	.4	6	.4	6	.3
22	6	.1	6	.2	6	.3	6	.2
30	6	.1	6	.1	6	.2	6	.2
40	6	.1	6	.1	6	.1	6	.1
Check	9	1.4	18	3.8	27	10.2	38	13.9
BORAX								
15	9	1.0	17	2.5	32	10.5	40	12.2
40	8	.5	25	3.0	36	9.8	39	8.6
80	8	.8	19	3.2	29	5.8	34	6.6
140	7	.3	18	2.6	23	3.6	31	5.6
220	7	.4	13	2.3	21	2.5	27	1.9
340	7	.2	10	.7	9	.3	9	.7
490	6	.2	8	.3	8	.3	8	.4
680	6	.1	7	.3	7	.2	7	.2
920	6	.1	6	.2	6	.1	6	.1
Check	14	1.2	18	3.0	34	9.9	45	13.1

The results with borax in water cultures resemble those in soils. Comparison with the concentrations in the water used for moistening the soils shows that toxicity has been reduced from about two times in the Fresno soil to approximately four times in the Stockton soil. This indicates a difference in availability in general agreement with the difference in degree of retention of these two chemicals shown in leaching tests (*4*, *8*).

Chlorate response in solution cultures is similar to that in soils, the toxicity being a function of nutrient level. The changes in toxicity of chlorate as affected by nutrient level, and the interrelations between them, indicate that water-soluble nutrients are the ones involved.

TOXICITY IN CHLORATE-CONTAINING SOIL CULTURES WITH SINGLE-SALT AMENDMENTS

The usual interpretation of the observed relation between soil fertility and chlorate toxicity (*6*) [6] is that the organic matter in the soil

[6] KIESSELBACH, T. A. REPORT OF MEETING OF REGIONAL WORKERS ON BINDWEED CONTROL, SPONSORED BY DIVISION OF CEREAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, U. S. DEPT. AGR. Ames, Iowa. 1936. [Mimeographed.]

reacts with the chlorate to reduce it to chloride. Since, however, the relation between fertility and toxicity holds in water cultures, phenomena other than chlorate reduction are apparently involved. To separate, if possible, the effects of the common nutrient ions, and to study the influence of each alone on chlorate toxicity, the experiments described under Methods (p. 639) and reported in table 6 were performed. This work involved 15 separate treatments on each of 10 different soils, the 9 listed in table 1 and Egbert loam, a semipeat from north of Rio Vista in the Sacramento River delta, a highly fertile soil requiring 170 cc of water per 500-g sample to moisten it to field capacity. Each treatment was represented by a series containing 10 soil cultures with the standard chlorate concentrations. The various ions used are given in table 6, the concentrations being listed on page 639.

TABLE 6.—*Effects of nutrient salts upon chlorate toxicity in 10 California soils as shown by growth of Kanota oats*

[Plants harvested in November and December 1936 and January 1937]

YOLO ADOBE CLAY

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2H		NO_3-K		NO_3-Na		NO_3-Ca		K-NO_3		K-Cl		K-SO_4		PO_4-KH_2		PO_4-NaH_2		PO_4-H_3		NO_3-NH_4		NH_4-NO_3		NH_4-Cl		NH_4-SO_4	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Cm	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*
5	18	1.6	33	10.3	32	7.7	34	9.3	33	7.8	30	5.9	26	3.4	25	3.0	27	4.0	26	3.0	27	4.1	34	8.7	35	9.0	34	7.8	35	9.1
15	20	2.3	34	9.2	36	7.7	31	8.0	33	7.9	28	6.0	23	2.5	23	2.7	22	2.5	25	2.6	26	3.4	33	8.9	32	7.9	35	7.2	33	6.8
40	14	1.2	32	8.2	32	6.7	29	7.1	35	6.3	28	4.7	10	1.4	8	1.1	12	1.0	16	1.4	12	1.2	35	8.7	30	5.2	28	3.9	28	3.9
80	8	.5	30	4.6	27	3.8	28	4.6	29	5.6	20	2.0	4	.3	5	.3	6	.5	7	.4	5	.3	30	6.1	27	3.2	10	1.0	10	1.3
140	5	.4	28	3.0	24	2.5	18	1.5	20	2.6	10	1.2	3	.2	4	.2	4	.2	4	.3	5	.3	28	4.7	13	1.6	4	.3	5	.4
220	4	.2	12	1.4	12	1.2	10	1.0	13	1.5	9	.5	3	.2	3	.2	3	.1	3	.2	4	.2	10	1.5	9	.7	3	.3	4	.2
340	3	.1	10	.9	7	.5	8	.9	10	1.2	6	.5	2	.2	2	.1	2	.1	2	.1	3	.2	9	.7	7	.5	2	.2	0	.0
490	0	.0	6	.5	6	.5	6	.7	10	.6	3	.2	2	.1	1	.1	0	.0	2	.1	2	.1	6	.5	4	.2	2	.1	0	.0
680	0	.0	6	.4	4	.4	6	.6	6	.4	3	.1	2	.1	0	.0	0	.0	0	.0	2	.1	4	.2	2	.1	0	.0	0	.0
Check	22	1.6	34	10.4	34	9.2	37	9.7	34	9.0	33	6.9	27	4.4	28	3.6	29	4.0	25	3.3	25	4.7	35	10.0	33	9.1	36	8.7	35	9.7

STOCKTON ADOBE CLAY

5	17	1.6	27	9.5	26	6.1	25	6.0	23	5.2	21	4.5	13	1.8	13	1.6	15	1.6	19	3.2	15	1.7	28	7.4	27	7.3	28	7.4	26	6.5
15	12	1.1	27	9.8	22	5.2	25	5.7	24	5.2	22	4.3	15	1.6	14	1.1	13	1.2	16	1.7	13	1.2	28	7.0	27	6.9	25	4.8	27	5.5
40	5	.3	27	10.1	23	4.8	23	5.4	21	4.8	23	3.3	9	.4	9	.3	5	.2	5	.1	6	.3	26	6.4	24	5.3	17	1.4	17	1.4
80	3	.1	27	5.5	19	2.8	23	3.4	25	3.3	12	1.3	4	.1	4	.1	3	.1	4	.1	4	.1	22	4.3	24	2.9	9	.4	6	.2
140	0	.0	23	3.0	21	1.9	19	2.1	18	2.3	8	.4	3	.1	0	.0	0	.0	4	.1	0	.0	17	2.1	11	.9	4	.2	3	.1
220	0	.0	5	1.3	10	.9	13	1.2	9	.7	5	.1	0	.0	0	.0	0	.0	0	.0	0	.0	15	1.2	10	.4	3	.1	3	.1
340	0	.0	4	.2	6	.4	9	.7	8	.4	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	11	.5	7	.2	0	.0	0	.0
490	0	.0	2	.1	6	.4	7	.2	4	.3	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	7	.2	0	.0	0	.0	0	.0
680	0	.0	0	.0	3	.2	4	.1	3	.2	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	5	.1	0	.0	0	.0	0	.0
Check	18	2.2	32	10.5	27	6.6	30	6.6	28	6.5	22	4.5	14	2.1	14	2.0	14	1.5	15	2.2	15	2.2	33	8.7	29	7.8	30	8.2	32	8.1

TABLE 6.—*Effects of nutrient salts upon chlorate toxicity in 10 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested in November and December 1936 and January 1937]

AIKEN CLAY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2H		NO_3–K		NO_3–Na		NO_3–Ca		K–NO_3		K–Cl		K–SO_4		PO_4–KH_2		PO_4–NaH_2		PO_4–H_3		NO_3–NH_4		NH_4–NO_3		NH_4–Cl		NH_4–SO_4	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Cm	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*
5	17	1.7	22	3.8	21	3.5	25	3.8	22	3.6	20	3.0	16	1.8	17	1.6	18	1.9	18	1.8	18	1.5	26	4.0	24	3.6	19	2.5	20	2.7
15	12	.7	23	3.5	21	3.5	23	3.2	19	3.4	19	2.6	15	1.0	13	.9	23	1.3	11	.9	10	.7	25	3.9	19	2.8	16	1.1	15	.8
40	5	.2	21	2.7	20	2.3	20	1.9	18	2.5	16	1.4	5	.3	7	.2	10	.6	7	.3	6	.3	22	2.9	15	1.4	10	.4	8	.2
80	0	.0	14	1.3	15	1.1	16	.8	13	1.2	9	.7	0	.0	0	.0	4	.2	3	.1	5	.1	16	1.5	11	.7	7	.1	4	.1
140	0	.0	10	.5	7	.5	11	.4	9	.7	5	.2	0	.0	0	.0	0	.0	0	.0	0	.0	12	.8	7	.3	0	.0	0	.0
220	0	.0	7	.3	7	.2	7	.2	7	.3	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	7	.3	5	.1	0	.0	0	.0
340	0	.0	6	.1	5	.1	5	.1	5	.2	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	6	.1	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0	3	.1	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0
Check	18	2.3	23	4.0	21	3.4	26	4.0	21	3.4	22	3.0	17	2.2	15	2.0	17	2.4	18	2.4	17	2.4	28	4.4	21	3.1	20	2.8	21	3.1

FRESNO SANDY LOAM

5	25	3.3	29	7.8	34	8.1	32	7.5	34	7.4	24	5.0	22	3.1	23	3.0	23	2.7	22	3.5	23	3.8	31	10.9	30	8.7	32	9.0	29	7.2
15	25	2.9	28	7.3	32	8.2	30	6.9	30	6.7	24	4.4	23	2.5	22	2.2	22	2.4	25	3.2	23	3.1	35	10.7	31	7.5	31	7.0	30	6.3
40	17	1.4	26	5.9	34	6.7	30	6.4	30	5.7	25	3.7	15	1.3	9	.6	11	1.0	14	1.2	12	1.1	32	8.4	30	5.5	30	4.8	25	4.4
80	9	.4	26	4.7	31	5.0	29	5.3	30	4.5	24	2.7	8	.3	5	.1	5	.2	4	.1	5	.3	27	5.3	29	4.4	25	3.7	21	2.0
140	5	.1	22	2.6	25	2.2	28	3.3	21	1.4	17	1.2	4	.2	0	.0	4	.1	3	.1	4	.1	25	3.0	21	1.9	15	1.4	13	.8
220	5	.1	19	1.4	16	1.0	10	.7	10	.9	8	.4	3	.1	0	.0	0	.0	0	.0	0	.0	14	1.1	14	.8	6	.5	6	.3
340	0	.0	12	.5	9	.2	7	.2	6	.1	5	.2	0	.0	0	.0	0	.0	0	.0	0	.0	13	.5	9	.4	5	.2	0	.0
490	0	.0	9	.2	6	.1	5	.1	5	.1	4	.1	0	.0	0	.0	0	.0	0	.0	0	.0	9	.3	7	.2	0	.0	0	.0
680	0	.0	3	.1	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	4	.1	0	.0	0	.0	0	.0
Check	21	3.4	31	7.4	33	8.2	33	8.1	32	7.3	27	5.2	22	3.4	23	3.1	19	2.8	22	4.6	21	4.3	34	10.0	31	9.1	30	9.7	28	7.9

ARBUCKLE CLAY LOAM

5	22	3.4	25	6.9	29	7.9	28	7.8	30	7.6	24	5.2	22	3.0	20	3.4	23	3.4	21	3.1	20	2.9	32	9.4	30	8.3	27	7.3	28	7.8
15	21	2.3	25	6.3	28	7.4	25	7.5	27	7.5	24	5.0	10	1.0	21	1.9	18	2.5	17	2.1	20	2.9	31	9.2	29	7.4	26	5.3	26	4.5
40	7	.4	25	6.2	27	7.1	23	6.0	25	6.3	21	2.8	10	.8	8	.8	7	.5	5	.3	9	.4	28	7.6	29	5.1	8	1.0	7	.4
80	4	.1	25	5.9	22	2.2	21	2.5	25	3.0	10	.7	6	.2	3	.1	4	.1	3	.1	4	.1	28	6.0	11	.8	5	.2	4	.1
140	3	.1	11	1.9	11	.6	10	.6	14	1.7	4	.3	3	.1	0	.0	0	.0	0	.0	0	.0	15	1.2	8	.3	3	.1	0	.0

220	0	.0	10	.8	7	.3	7	.3	9	.5	3	.2	0	.0	0	.0	0	.0	0	.0	0	.0	8	.3	4	.2	0	.0	0	.0
340	0	.0	5	.1	5	.1	4	.1	5	.2	2	.1	0	.0	0	.0	0	.0	0	.0	0	.0	4	.1	3	.1	0	.0	0	.0
490	0	.0	3	.1	0	.0	0	.0	3	.1	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	3	.1	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0
Check	23	3.6	30	8.7	29	8.4	28	7.8	30	8.1	26	5.6	24	3.5	22	3.3	22	3.2	21	3.6	23	3.3	30	10.0	30	8.1	28	7.8	30	8.4

YOLO FINE SANDY LOAM

5	26	6.7	28	8.5	28	7.7	28	7.4	29	8.8	27	8.0	28	7.1	27	7.3	28	6.8	27	6.9	27	7.2	34	9.8	32	8.0	31	7.5	30	9.5
15	25	5.7	30	8.2	30	7.1	27	6.4	29	7.3	28	7.7	27	6.9	27	6.6	27	5.7	25	6.0	28	6.4	31	10.3	32	8.5	27	8.1	35	7.6
40	23	3.2	29	7.7	28	6.0	27	5.9	24	6.4	24	4.9	23	3.3	25	3.6	24	3.9	25	3.4	24	2.8	29	7.7	32	7.1	28	7.0	31	7.6
80	20	1.4	25	4.3	26	4.2	22	3.5	24	3.9	14	1.3	20	1.8	12	.9	19	1.8	18	1.9	22	2.1	23	3.5	29	4.5	23	2.7	24	3.5
140	10	.7	22	2.5	23	2.6	18	2.2	20	2.3	9	.6	13	.6	6	.2	8	.5	8	.6	11	1.1	22	2.3	23	2.8	15	1.3	16	1.4
220	6	.3	13	1.0	12	1.4	9	.8	13	.9	9	.6	6	.3	5	.1	5	.2	4	.1	7	.2	14	1.3	10	.8	3	.1	3	.2
340	4	.1	6	.3	8	.7	7	.4	7	.3	4	.2	0	.0	4	.1	4	.2	3	.1	0	.0	9	.6	8	.4	3	.1	3	.1
490	0	.0	4	.2	7	.5	4	.3	4	.1	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	7	.3	4	.2	0	.0	0	.0
680	0	.0	4	.2	2	.4	3	.1	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	3	.1	0	.0	0	.0	0	.0
Check	30	7.7	29	8.7	31	7.8	28	7.5	32	8.6	32	8.0	28	6.8	30	6.9	27	6.6	28	7.2	27	7.4	32	11.9	32	9.3	31	8.5	34	10.1

EGBERT LOAM

5	27	8.1	34	10.3	28	7.6	28	7.2	32	8.1	27	7.9	27	7.5	27	7.2	28	7.9	31	9.4	32	9.5	33	8.7	31	9.0	32	8.3	32	8.7
15	28	8.2	32	9.0	28	8.1	30	8.3	33	9.5	28	8.6	29	7.3	29	8.2	27	7.0	32	10.2	31	9.0	34	8.6	28	8.6	31	8.4	33	9.0
40	24	5.7	28	7.9	28	7.2	28	7.0	29	8.1	26	7.2	27	6.0	26	6.1	27	4.4	28	6.3	30	7.3	29	7.8	29	7.7	28	6.7	33	7.8
80	16	1.5	30	7.7	27	5.9	27	6.1	26	6.4	27	6.5	29	5.1	21	2.8	17	1.3	23	2.5	23	2.7	29	6.9	24	5.5	25	4.6	26	4.3
140	7	.4	27	5.4	24	4.0	25	4.3	25	5.2	15	1.4	10	1.0	8	.4	8	.4	8	.5	10	.7	27	5.8	22	3.0	11	1.0	9	1.0
220	4	.1	10	3.2	18	1.1	18	1.6	19	1.8	7	.6	7	.3	5	.2	4	.1	6	.3	6	.2	25	3.1	7	.5	5	.3	8	.3
340	0	.0	7	.3	8	.4	7	.4	9	.5	5	.2	4	.1	0	.0	0	.0	3	.2	3	.2	11	.8	6	.3	4	.3	4	.2
490	0	.0	4	.2	5	.2	4	.1	5	.2	5	.1	0	.0	0	.0	0	.0	3	.1	2	.1	5	.3	4	.2	3	.2	0	.0
680	0	.0	4	.1	4	.1	3	.1	4	.1	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	4	.2	3	.1	0	.0	0	.0
Check	30	8.2	33	10.0	31	9.0	30	8.5	32	7.6	27	8.4	29	7.5	28	7.5	28	7.6	32	10.2	31	9.3	30	8.5	29	6.9	31	8.8	34	9.5

COLUMBIA FINE SANDY LOAM

5	30	9.2	32	10.1	36	13.5	33	12.8	35	12.9	30	9.8	33	10.4	29	8.0	30	9.0	32	9.6	32	10.1	34	13.0	32	10.8	32	10.9	33	10.5
15	31	9.1	32	10.8	37	12.4	35	13.4	34	12.9	32	10.5	32	10.1	31	9.1	29	8.3	30	9.8	31	11.1	33	10.9	32	10.6	33	10.7	30	10.0
40	32	8.1	31	9.8	36	11.4	35	12.2	36	10.9	32	9.9	34	10.0	31	7.4	32	7.0	31	8.3	33	8.9	32	10.5	32	7.3	31	7.5	31	6.9
80	27	5.2	31	8.0	35	10.8	34	10.0	34	10.9	31	8.3	31	6.7	28	5.2	26	4.0	27	4.1	29	6.5	29	6.0	26	4.2	23	3.4	23	2.5
140	15	2.2	30	6.6	31	8.0	30	6.4	31	7.7	25	4.8	23	3.4	10	1.0	8	.7	17	2.2	23	1.2	26	3.7	22	2.0	15	1.6	9	1.0
220	9	.7	23	3.2	27	5.9	27	4.7	24	3.5	21	2.4	15	1.5	7	.5	3	.2	9	.8	8	.6	20	2.3	10	1.2	10	.9	7	.6
340	2	.1	12	1.4	14	1.1	9	.6	13	1.0	10	.8	8	.5	0	.0	0	.0	5	.3	5	.3	13	1.1	8	.7	5	.3	5	.4
490	0	.0	8	.3	7	.5	8	.3	10	.5	4	.3	4	.3	0	.0	0	.0	2	.2	3	.1	8	.6	5	.3	4	.2	4	.2
680	0	.0	5	.2	6	.3	7	.3	3	.2	3	.2	0	.0	0	.0	0	.0	0	.0	0	.0	6	.4	3	.2	4	.2	3	.1
Check	29	8.8	30	10.7	36	12.2	35	12.6	34	13.5	32	11.7	30	8.7	29	8.3	30	8.9	30	9.8	26	10.3	35	14.1	34	11.3	35	12.5	29	10.5

TABLE 6.—*Effects of nutrient salts upon chlorate toxicity in 10 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested in November and December 1936 and January 1937]

YOLO CLAY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2H		NO_3-K		NO_3-Na		NO_3-Ca		K-NO_3		K-Cl		K-SO_4		PO_4-KH_2		PO_4-NaH_2		PO_4-H_3		NO_3-NH_4		NH_4-NO_3		NH_4-Cl		NH_4-SO_4	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Cm	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*
5	29	6.6	29	11.7	37	10.5	37	10.6	34	8.2	32	9.2	34	11.4	32	10.3	31	9.6	33	9.9	32	9.7	35	14.0	32	10.7	36	14.8	35	10.8
15	30	6.1	29	10.7	37	10.1	36	9.5	33	8.1	32	10.2	32	9.2	33	10.1	33	8.8	33	9.2	35	9.3	31	12.4	32	10.1	35	13.8	35	10.2
40	27	4.0	32	10.0	34	9.3	35	9.8	33	8.1	31	7.8	33	7.5	30	8.7	32	7.9	32	6.6	31	8.9	31	9.8	30	9.2	35	10.2	33	8.6
80	23	2.9	32	8.4	34	8.0	35	8.5	30	7.2	30	6.4	30	6.7	28	5.1	13	1.3	27	3.7	20	1.4	28	7.7	27	6.7	28	5.8	29	5.0
140	14	1.7	31	7.2	30	6.7	32	6.3	28	5.1	25	4.2	23	3.1	10	1.2	10	.7	9	1.5	8	.4	27	5.5	26	4.7	26	4.6	15	4.3
220	9	.9	28	5.8	29	4.9	29	5.2	27	4.3	23	3.1	8	1.4	5	.4	6	.3	6	.2	3	.2	26	4.9	22	3.5	21	2.6	19	2.1
340	7	.3	22	2.8	20	1.5	23	3.4	23	3.2	9	.7	10	1.1	5	.3	5	.2	0	.0	3	.2	21	2.8	11	1.2	15	1.3	15	1.3
490	7	.2	9	.9	15	1.1	15	1.7	17	1.2	9	.7	9	.8	3	.1	3	.1	0	.0	2	.1	15	1.5	9	1.0	7	.7	7	.7
680	4	.1	9	.6	6	.5	9	.9	7	.7	6	.6	6	.3	3	.1	0	.0	0	.0	0	.0	8	.8	6	.3	7	.4	6	13.6
Check	33	9.3	33	11.0	35	10.4	34	10.6	34	10.1	31	11.5	33	11.4	33	11.0	32	10.2	30	9.9	32	9.7	34	17.1	35	11.5	35	15.1	33	.4

SACRAMENTO CLAY

5	37	11.0	38	11.6	43	11.2	45	11.5	42	10.8	37	10.2	36	9.8	39	9.7	41	11.6	35	10.3	38	10.4	41	11.0	41	10.7	42	10.3	45	10.7
15	35	10.0	40	10.8	41	11.2	44	11.0	39	10.3	33	9.8	36	9.7	38	9.3	40	11.0	34	9.4	36	8.5	44	10.7	38	10.6	42	9.4	41	9.3
40	33	7.5	39	10.1	43	9.9	39	9.7	35	9.8	31	8.9	35	7.3	36	7.8	40	9.4	32	8.7	35	7.4	46	10.0	40	9.8	38	7.3	40	8.2
80	30	4.8	38	8.8	34	6.7	37	7.6	28	7.0	28	6.2	29	4.9	30	4.7	37	6.8	31	6.0	29	5.0	41	8.8	36	8.0	31	4.0	28	4.7
140	21	2.4	32	6.0	31	4.1	31	5.2	27	4.5	23	3.4	25	2.7	25	2.3	30	4.2	26	3.3	22	2.8	40	6.7	26	5.0	22	1.9	21	1.8
220	16	1.3	25	3.5	25	2.9	31	3.5	22	2.8	18	1.9	17	1.1	17	1.1	21	2.2	21	1.7	20	1.6	36	4.8	19	2.5	15	1.0	17	.9
340	10	.9	16	2.0	23	2.0	26	2.1	27	1.7	15	1.1	9	.5	11	.5	20	1.0	10	1.0	8	1.0	25	3.2	10	1.1	8	.4	8	.3
490	6	.4	12	1.0	13	1.0	14	1.1	13	1.0	11	.7	4	.2	5	.2	9	.5	10	.5	8	.5	19	1.6	11	.5	5	.3	3	.1
680	5	.1	8	.5	12	.3	13	.8	8	.6	9	.3	0	.0	0	.0	3	.2	6	.3	5	.2	14	.8	6	.4	0	.0	0	.0
Check	37	11.7	42	11.0	40	11.0	42	13.2	42	10.9	37	10.3	35	9.6	36	9.5	41	11.8	35	11.5	39	10.0	44	12.5	37	10.0	45	10.8	39	10.6

In addition to the data of table 6, the results for certain salts in Yolo clay loam, Stockton adobe clay, and Fresno sandy loam are plotted in figures 9, 10, and 11.

The nitrate curves showed no significant differences between the various salts, the concentration of nitrate being the important factor. In the graphs, consequently, the nitrate values for potassium, sodium, and calcium salts were averaged and the average values plotted. In

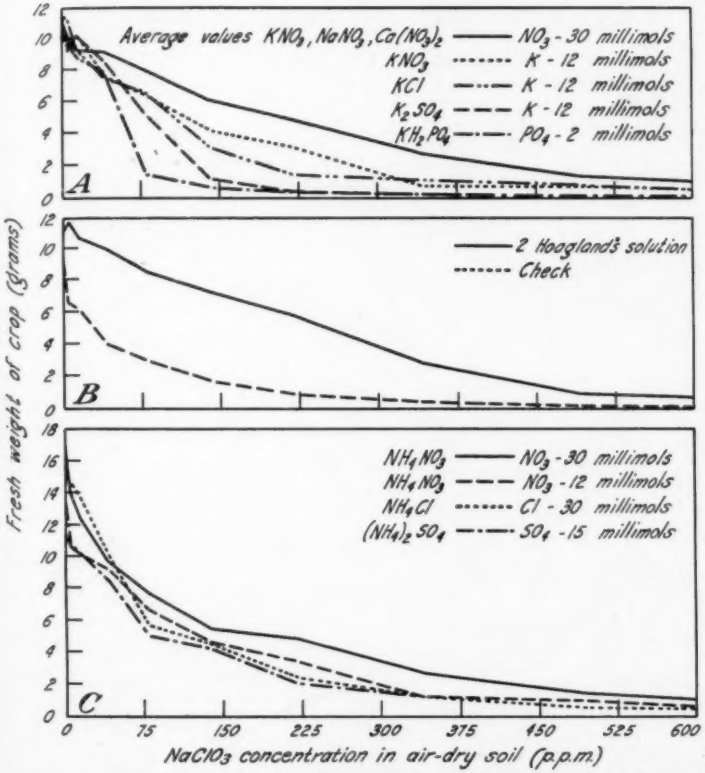
Average values KNO_3, $NaNO_3$, $Ca(NO_3)_2$ — NO_3 - 30 millimols
KNO_3 ····· K - 12 millimols
KCl —·— K - 12 millimols
K_2SO_4 – – – K - 12 millimols
KH_2PO_4 —··— PO_4 - 2 millimols
A
2 Hoagland's solution
Check
B
NH_4NO_3 — NO_3 - 30 millimols
NH_4NO_3 – – – NO_3 - 12 millimols
NH_4Cl ····· Cl - 30 millimols
$(NH_4)_2SO_4$ —··— SO_4 - 15 millimols
C
Fresh weight of crop (grams)
0 75 150 225 300 375 450 525 600
$NaClO_3$ concentration in air-dry soil (p.p.m.)

FIGURE 9.—Relation of single-salt applications to chlorate toxicity in Yolo clay loam: *A*, Results of applying various potassium, sodium, and calcium salts individually at concentrations equivalent to those used in Hoagland's solution; *B*, check culture results with chlorate in tap water and in 2H solution; *C*, results with several ammonium salts applied individually.

all cases the toxicity in the nitrate series containing 30 millimols per liter corresponds closely to that in the 2H series containing the same nitrate concentration. Though the absolute values in the Stockton soil are not the same, probably because some other element is further stimulating growth in the 2H series, the values relative to the checks are very similar.

There is a close relation between nitrate level and chlorate toxicity; the higher the nitrate level, the lower the toxicity. In the potash series in the Yolo clay loam (fig. 9) there is apparently a definite relation between the anions present. Nitrate reduced chlorate toxicity to the greatest extent, followed in order by chloride, sulphate, and phosphate. It has been commonly observed that water-culture

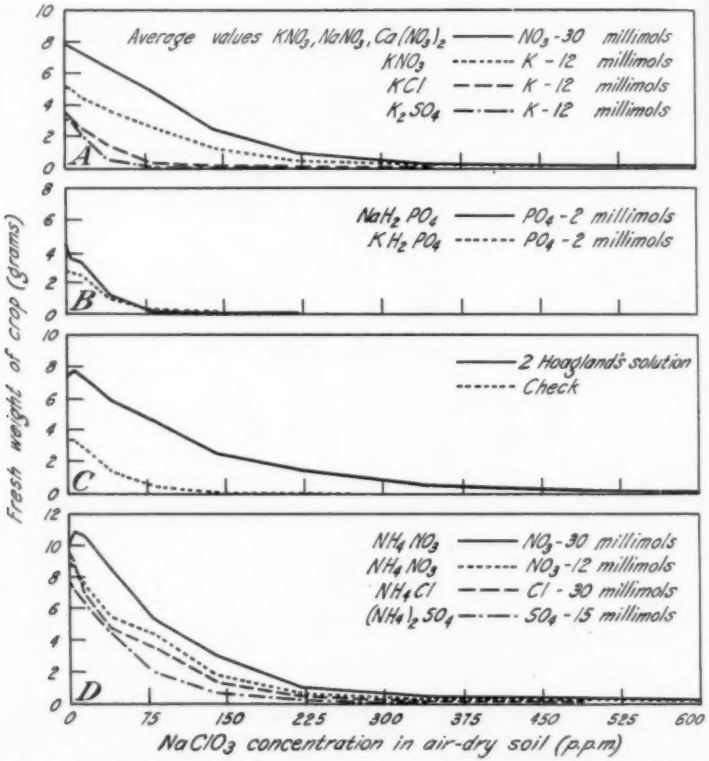
Average values KNO_3, $NaNO_3$, $Ca(NO_3)_2$ —— NO_3 - 30 millimols
KNO_3 ------- K - 12 millimols
KCl – – – K - 12 millimols
K_2SO_4 —·— K - 12 millimols
A
NaH_2PO_4 —— PO_4 - 2 millimols
KH_2PO_4 ------- PO_4 - 2 millimols
B
—— 2 Hoagland's solution
------- Check
C
NH_4NO_3 —— NO_3 - 30 millimols
NH_4NO_3 ------- NO_3 - 12 millimols
NH_4Cl – – – Cl - 30 millimols
$(NH_4)_2SO_4$ —·— SO_4 - 15 millimols
D
Fresh weight of crop (grams)
0 75 150 225 300 375 450 525 600
$NaClO_3$ concentration in air-dry soil (p.p.m)

FIGURE 10.—Relation of single-salt applications to chlorate toxicity in Stockton adobe clay: *A*, Results of applying various potassium, sodium, and calcium salts individually; *B*, results of applying the phosphates individually; *C*, controls with chlorate in tap water and in 2H solutions; *D*, results with several ammonium salts used individually.

plants absorb these anions in this same decreasing order. Evidently chlorate toxicity is related to anion absorption by the plant.

In the Yolo clay loam all the ammonium salts reduced the chlorate toxicity somewhat as compared with the tap-water check. About a week elapsed between planting and the first inception of chlorate effects in the intermediate toxicity range in these cultures. The plants evidently required some time to absorb a toxic dose of chlorate, and this absorption did not become evident until the first leaves

unfolded, except in the very high concentrations. During these days, in the Yolo soil, nitrification of the ammonia evidently took place (*2*) and toxicity declined. Among the ammonium salts the anion relation may be noted in the intermediate toxicity range but is less pronounced than among the potassium salts.

The Yolo clay loam used in these tests is a heavy soil of high fertility. In the graphs of the Stockton adobe clay it may be noted from the check curves showing series moistened with tap water and with double-strength Hoagland's solution that the Yolo soil initially had nearly as

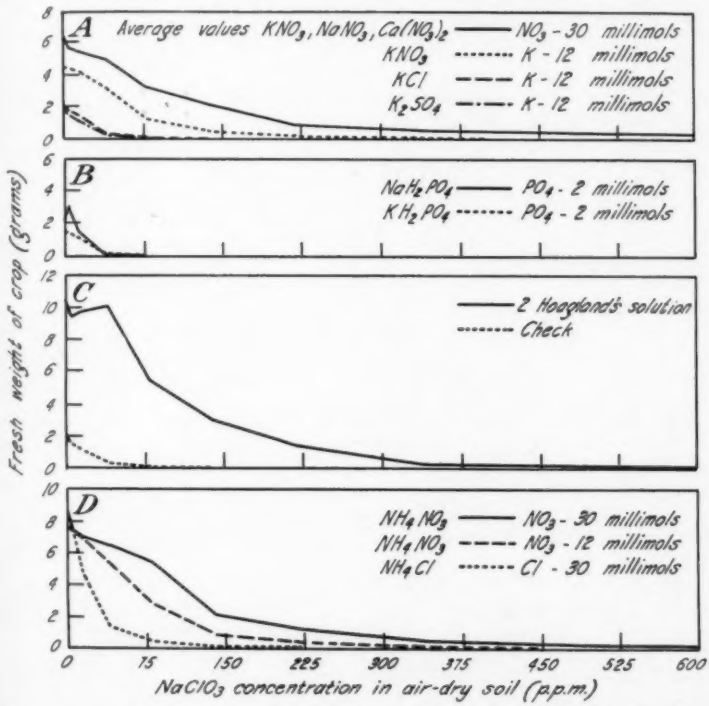
A
Average values KNO_3, $NaNO_3$, $Ca(NO_3)_2$ —— NO_3 - 30 millimols
KNO_3 ····· K - 12 millimols
KCl – – – K - 12 millimols
K_2SO_4 –·–· K - 12 millimols
B
NaH_2PO_4 —— PO_4 - 2 millimols
KH_2PO_4 ····· PO_4 - 2 millimols
C
—— 2 Hoagland's solution
····· Check
D
NH_4NO_3 —— NO_3 - 30 millimols
NH_4NO_3 – – – NO_3 - 12 millimols
NH_4Cl ····· Cl - 30 millimols
Fresh weight of crop (grams)
0 75 150 225 300 375 450 525 600
$NaClO_3$ concentration in air-dry soil (p.p.m.)

FIGURE 11.—Relation of single-salt applications to chlorate toxicity in Fresno sandy loam: *A*, Results of applying potassium, sodium, and calcium salts individually; *B*, results of applying the phosphates individually; *C*, controls with chlorate in tap water and in 2H solutions; *D*, results with several ammonium salts used individually.

much nitrate as did the Stockton soil after it was moistened with the Hoagland's solution. Moistened only with tap water, the Stockton soil produced a very low yield, and toxicity was extremely high, as has been noted before (*4*).

In consequence of the low initial fertility, the yields of the check plants in the Stockton soil (fig. 10) correlate closely with nitrate application, as does chlorate toxicity. It is evident from these curves that potassium concentration has little or no effect upon either crop

yield or chlorate toxicity. In this soil of low fertility, furthermore, the nitrate effects far outshadow those of other anions, and the chloride, sulphate, and phosphate curves are not distinctly different.

The curves for the ammonium salts in the Stockton soil differ materially from those of the Yolo clay loam. Though the series show lowering of toxicity related to the two nitrate levels, the chloride and sulphate curves, which, being almost identical, are represented only by the chloride results, show no effect of the ammonium ion upon toxicity. If these curves are compared with the tap-water check and if the results from both are expressed as percentage of the chlorate-free cultures, they will prove to be almost identical. This fact indicates that nitrification in this soil has proceeded slowly, so that the toxic effects of the chlorate had become permanent before any appreciable amount of nitrate was developed. Slow nitrification during the later life of the plants stimulated growth in the lower toxicity range without altering the original toxicity relation. Again, the nitrate effects overshadowed other anions, and no differences due to chloride or sulphate were found.

The Fresno sandy loam is a coarse soil holding less than half as much water at field capacity as the Stockton soil. Since the chlorate is applied in solution with the concentrations based on the air-dry soil, the toxicity should be higher in the Fresno than in the Stockton soil. Examination of the curves (fig. 11), however, shows it to be somewhat lower. Noting the crop yield in the tap-water checks with no chlorate, it is seen that the Fresno soil is somewhat the more fertile of the two; and this fact evidently explains the lower toxicity. As compared with the nitrate level in the soil, water-holding capacity seems to affect toxicity very little.

Though the anion relation in the Fresno soil is in the same direction as in the Yolo, it is less pronounced. This, again, seems to be the case where nitrate effects overshadow those of other ions in soils of low fertility. Among the ammonium salts toxicity has been appreciably reduced by the chloride and sulphate, a fact indicating a more rapid nitrification in this soil, though not so rapid as in Yolo clay loam.

Summarizing the results in the 10 soils from a study of graphs prepared in the analysis of the data, it is evident that nitrate ion has the predominating influence among common soil nutrients in reducing chlorate toxicity. The correlation between the nitrate curves and those for the double-strength Hoagland's solution is evident in all 10 soils. The relation between nitrate level and toxicity reduction is evident from a study of the two levels in the potassium and ammonium salts. Chlorate toxicity is apparently a function of nitrate content in these soils, and any addition of nitrate further reduces toxicity. A single exception exists in the Yolo fine sandy loam, where no distinct difference is shown between the two concentrations of ammonium nitrate added. Perhaps in this highly fertile soil nitrification of the ammonium ions has thrown the results out of line.

Considering next the relation of chloride and sulphate concentration to chlorate toxicity, so evident in the Yolo clay loam, this effect is found to be most pronounced in the fertile soils that show no great response in crop production to fertilization. This tendency for chloride to predominate over sulphate in its effect on chlorate toxicity

is very pronounced in the potassium salts in Columbia fine sandy loam, Yolo clay loam, and Egbert loam. It is definitely present in Sacramento clay, Fresno sandy loam, and Aiken clay loam. Among the ammonium salts it is evident in Columbia fine sandy loam, Yolo clay loam, Fresno sandy loam, Arbuckle clay loam, and Aiken clay loam. In the other soils this effect with these salts is either less pronounced or inconsistent. In no case is the relation consistently reversed.

In three of the more fertile soils phosphate ion seems definitely less effective than sulphate in reducing chlorate toxicity; in four soils, including two fertile and two relatively infertile, it seems more effective; and in the three least fertile it shows no consistent relationship. Apparently, therefore, it would be impossible at present to state the effect that phosphate concentration might have on chlorate toxicity, and it would be dangerous to assume, as might be inferred from the results on Yolo clay loam alone, that phosphate increased chlorate effects. One should bear in mind that the phosphate applications in these tests were low as compared with the other nutrients added. To find the effects of these various ions more definitely, it would be desirable to add them in equal concentration instead of in the amounts actually required by the plants. With phosphate this procedure would introduce the problems of solubility and availability. Such studies lie outside the scope of the present work.

Careful analysis of the data on these soils indicates that the cations involved had no primary effect upon chlorate toxicity. The nitrates of potassium, sodium, and calcium were equally effective and showed no consistent cation relationship. Ammonium ion as such had no effect, but when added to soils of high nitrifying power the resulting nitrate was effective. Whether the product of this nitrification affected the toxicity depended on the relative rates of nitrification and chlorate absorption.

The highest nitrification rates occurred in Egbert loam and Yolo fine sandy loam. In these soils ammonium chloride and sulphate materially reduced chlorate toxicity. In Yolo clay loam and Fresno sandy loam smaller changes occurred. In these four soils nitrification of ammonia proceeded so rapidly that nitrate was produced before the plants absorbed enough chlorate to be permanently injured. In the remaining six soils nitrification was not rapid enough to forestall the toxic effects of chlorate; and though crops in five of them were increased, a comparison made on the basis of the checks revealed no change in toxicity. In the Aiken soil there was no evidence of nitrification and the effects of the chloride and sulphate of ammonia differed in no essential feature from those of the potassium salts.

TOXICITY IN CHLORATE-CONTAINING WATER CULTURES WITH SINGLE-SALT AMENDMENTS

Again, to find the nature of soil effects in these studies, water cultures were established as described on page 640. Data on this experiment are presented in table 7 and plotted in figure 12.

The results in culture solution do not differ essentially from those in soils. Since the plants were not large in the single-salt cultures, and since chlorate toxicity was greatly reduced by the higher nitrate concentrations used, the scale has been altered somewhat in figure 12. In calcium nitrate solution containing 60 millimols per liter (equiva-

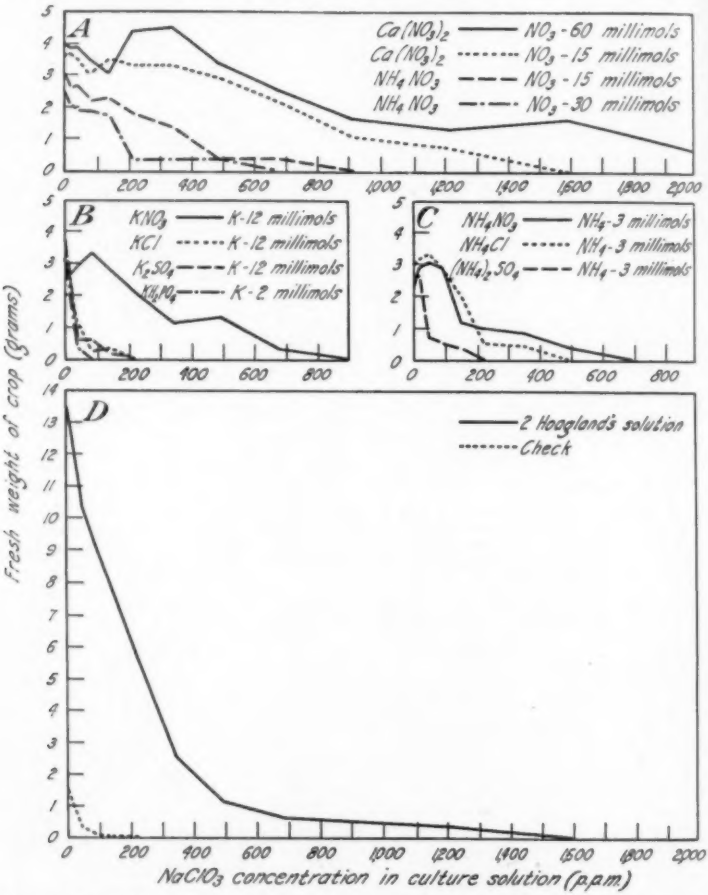
A
$Ca(NO_3)_2$ — NO_3 - 60 millimols
$Ca(NO_3)_2$ ····· NO_3 - 15 millimols
NH_4NO_3 – – – NO_3 - 15 millimols
NH_4NO_3 –·–·– NO_3 - 30 millimols
B
KNO_3 — K - 12 millimols
KCl ····· K - 12 millimols
K_2SO_4 – – – K - 12 millimols
KH_2PO_4 –·–·– K - 2 millimols
C
NH_4NO_3 — NH_4 - 3 millimols
NH_4Cl ····· NH_4 - 3 millimols
$(NH_4)_2SO_4$ – – – NH_4 - 3 millimols
D
— 2 Hoagland's solution
····· Check
Fresh weight of crop (grams)
$NaClO_3$ concentration in culture solution (p.p.m)

FIGURE 12.—Chlorate toxicity in various single-salt water cultures. The ion concentrations compare with those in the double-strength Hoagland's solution. *A*, Solutions containing calcium and ammonium nitrates; *B*, solutions containing potassium salts; *C*, solutions containing ammonium salts; *D*, controls with chlorate in tap water and in 2 H solution.

lent to approximately 4,000 p. p. m. of NO_3), plants survived at a chlorate concentration of 2,040 p. p. m. In the ammonium nitrate culture of this concentration the plants were killed. Even in the lower concentrations some toxicity was evident.

TABLE 7.—*Relation of nutrient salts to chlorate toxicity in water cultures as shown by growth of Kanota oats*

[Plants harvested Apr. 30, 1937. Nutrient-salt concentrations expressed in millimols per liter]

$NaClO_3$ in culture solution (p.p.m.)	Checks				$Ca(NO_3)_2$				NH_4NO_3				KNO_3		KCl		K_2SO_4		KH_2PO_4		NH_4NO_2		NH_4Cl		$(NH_4)_2SO_4$	
	Water		2H		7.5		30		15		30		12		12		6		2		3		3		1.5	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Cm	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*
15	12	1.0	35	12.5	21	3.7	22	3.8	13	2.5	13	2.0	13	2.6	16	2.6	15	2.7	13	2.1	16	2.9	18	3.2	17	2.7
40	9	.3	34	10.3	21	3.5	20	3.8	13	2.6	13	1.8	13	3.0	15	1.2	13	1.7	12	.4	16	3.1	18	3.4	13	.8
80	8	.2	34	9.1	18	3.0	22	3.5	12	2.2	13	1.8	13	3.4	12	.3	12	.7	0	.0	13	2.9	13	2.9	12	.6
140	5	.1	33	7.7	19	3.5	19	3.0	12	2.3	13	1.7	13	2.8	12	.4	9	.2	0	.0	12	1.2	13	2.1	11	.5
220	0	.0	27	5.6	20	3.3	27	4.4	12	1.8	12	.4	13	2.1	0	.0	0	.0	0	.0	12	1.0	12	.6	0	.0
340	0	.0	20	2.0	19	3.3	25	4.5	11	1.4	12	.4	12	1.2	0	.0	0	.0	0	.0	11	.9	11	.5	0	.0
490	0	.0	16	1.1	15	2.9	20	3.4	11	.4	12	.4	13	1.4	0	.0	0	.0	0	.0	9	.4	0	.0	0	.0
680	0	.0	11	.6	15	2.1	17	2.5	11	.4	0	.0	10	.4	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0
920	0	.0	10	.5	12	1.0	14	1.6	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0
1,220	0	.0	8	.4	10	.7	13	1.3	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0
1,590	0	.0	0	.0	0	.0	12	1.6	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0
2,040	0	.0	0	.0	0	.0	8	.5	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0
Check	12	1.5	36	13.5	21	3.6	28	4.0	13	3.0	14	2.5	13	2.5	16	2.5	17	3.9	18	3.2	15	2.3	17	3.0	19	2.7

Though the toxicity relation between the nitrate, chloride, and sulphate salts seems definite in the ammonium cultures, it is less distinct in the potassium solutions. The phosphate solution showed the highest toxicity of all the single-salt cultures. In viewing these results, however, one should remember that the plants in none of these single-salt cultures were vigorous; and deficiency symptoms in many cases overshadowed chlorate effects. The one outstanding contribution of this final study is the substantiation of the predominating nitrate effect. No matter how fascinating the anion interrelations may be, the practical aspect of all this work is that chlorate toxicity in soils is largely determined by nitrate concentration; and where chlorates are used for weed control the inherent nitrate-supplying power of the soil is of primary importance.[7]

DISCUSSION

With the relation between chlorate toxicity and nitrate concentration so definitely established, the natural inquiry is, How is this effect produced in the plant? Work on this problem is in progress, and eventually the mechanism should be worked out. Meanwhile the results of two preliminary experiments will suffice to answer a major question. Plants of Hubbard squash (*Cucurbita maxima* Duchesne) growing in aerated culture solutions were used to study chlorate absorption under conditions of high and low nitrate in the culture solutions. Plants were grown in each of two tanks, one containing a full Hoagland's solution, the other a one-tenth Hoagland's solution. After about 4 weeks the plants were cut off just below the cotyledons, rubber tubes were slipped over the ends, and xylem exudate was collected after the method of Hoagland.[8] Chlorate was then added to each tank to give concentrations of 100 p. p. m. in each tank, and the chlorate concentrations in successive samples of xylem exudate were determined for about 48 hours. It was found that in the high-nitrate culture little chlorate was absorbed from the solution, whereas in the low-nitrate culture chlorate ions were not only absorbed but concentrated in the xylem sap to many times the concentration of the external culture solution. The effect of nitrate in the culture medium is evidently to inhibit chlorate absorption by roots.

A careful study of the data relating to the general anion relationship indicates that chloride and sulphate ions affected chlorate absorption in all soils, but that the differences were large only in the more fertile soils, where high nitrate content shifted the critical toxicity range into the higher chlorate concentrations. Apparently a relation exists among the anions entering the plant, the probability of any one ion's absorption being determined by its tendency to enter the root as compared with the tendency of all other ions to enter. Since the tendency of one ion to enter would depend upon its concentration and its penetrability, apparently both the physiological activity of the root and the physical and chemical characteristics of the ion are involved. If this assumption is correct, the ability of any one ion to

[7] Just as this paper was ready to submit for publication, the writer's attention was called to a short article by Haines (*10*), who found that both inoculation and ammonium sulphate application improved the percentage survival of red clover seedlings in chlorate-treated soils. Both these treatments evidently increased the nitrate available to the seedlings.

[8] HOAGLAND, D. R., and BROYER, T. C. CERTAIN INTERRELATIONSHIPS BETWEEN ABSORPTION OF SALTS, ROOT PRESSURE, AND TRANSLOCATION OF SALTS. Paper read before the meeting of the American Association for the Advancement of Science, Seattle, Wash. 1936.

inhibit absorption of another would be related to both these functions, and conditions would determine which predominated. Considering the nitrate, chloride, and sulphate of potassium in the soil cultures, we find the added anion concentration in each case the same; the effect therefore results from the greater absorption of nitrate by the plant. As for the two nitrate levels of the potassium and ammonium salts, the greater tendency to enter, in this case, depends primarily upon the greater concentration of nitrate at the high levels. Consequently, among the anions present in the soil, nitrate would seem to have the predominating effect because it is readily absorbed; and among soils, those having high nitrates show lower chlorate toxicities because nitrate tends to enter the plants from the more concentrated soil solutions to the exclusion of chlorate.

That salts other than nitrates may also be involved seems evident from some results obtained on a Wyoming soil particularly high in chlorides and sulphates. Chlorates were reported to have had little effect on plants growing in this soil. A toxicity experiment in the greenhouse proved that this soil had an extremely low chlorate toxicity even though its fertility was not high. Saline soils therefore appear to respond to chlorate applications about as do highly fertile ones, apparently because the plants are unable to absorb chlorates from them.[9]

As these experiments emphasize, absorption of ions is purely a mechanical phenomenon, and utilitarian aspects of this function are secondary. It has long been known that plants will absorb and accumulate large amounts of bromide ion for which they have no use. It now appears that in the absence of nitrate they will absorb chlorate against a concentration gradient and accumulate it to their own detriment.

These results, considered in relation to field experience, clear up several controversial points. (1) Nitrate effects seem to outweigh concentration effects resulting from differences in moisture-holding capacity. For this reason heavy adobe soils, such as the Stockton soil used in these experiments, may show a high toxicity from chlorate, whereas a light soil, such as the Columbia fine sandy loam, may be low.

(2) Organic matter may relate to chlorate toxicity more directly through nitrate effects than through decomposition as has been postulated. Since chlorate has been known to persist in California soils for 3 years or more in semiarid areas and to disappear in wet areas within a year, leaching has apparently more to do with the disappearance of chlorate than has decomposition (*13*). The behavior just reported occurs irrespective of the organic-matter content of the soils in question.

(3) It has been shown here (*3*) that chlorate is more effective during the fall and spring than in summer. A previous publication (*1, p. 34*), in fact, asserted that chlorate applications should—

> aim to provide a lethal concentration of chlorate in the soil solution during the spring for absorption by the roots during the period when they are depleted of organic foods and engaged in rapid absorption from the soil.

[9] A previous paper reported the antagonistic action of borate upon chlorate toxicity (*7*). Probably all anions in the culture medium have some effect, the comparative importance of any one being related to its tendency to enter the plant.

Probably the nitrate content of the soil as affected by leaching during the winter is as vitally concerned as are organic reserves and absorption rate (*5*). Since a definite time is required for absorption and killing by chlorate, not only the content at one time but also the nitrate-supplying power of the soil is involved. Considering the seasonal cycle of nitrification and nitrate absorption by plants, spring seems in all ways the best time for killing with chlorate.

Even more immediate application of the nitrate-chlorate relation may be made. Though a few soils have definite mineral deficiencies, the fertility of most agricultural soils, as shown by vegetative growth of plants, is a reflection of nitrate content. Consequently, the response to chlorate applications may be predicted on most soils by a casual survey of the growing crops and roadside weeds. Although much experience will be required, and such matters as rainfall, species tolerance, and known mineral deficiencies must be considered, this method, when used for prescribing dosages, will eliminate considerable guesswork from chlorate application. In highly fertile soils, furthermore, costly experimentation with chlorate may be avoided and more reliable results obtained with a minimum waste of time by turning immediately to carbon bisulphide or to a cultural or cropping program.

SUMMARY

Although arsenic and boron toxicity are related primarily to textural grade of soils, chlorate toxicity is correlated with fertility.

In a number of soils, increasing the mineral nutrient level invariably decreased chlorate toxicity.

Water cultures of different nutrient levels showed a similar response.

Nutrient level did not affect arsenic or boron toxicity in the high application range either in soils or in water cultures.

In single-salt soil-culture series containing different concentrations of chlorate, those with added nitrate invariably showed lowered chlorate toxicity.

In certain cultures in the more fertile soils, chlorides and sulphates also reduced the toxicity somewhat.

Cations in the culture medium apparently did not affect chlorate toxicity. There was evidence that ammonium ions were nitrified so rapidly in some soils as to affect chlorate toxicity. In most soils, however, nitrification occurred, but went on too slowly to alter the chlorate toxicity.

Single-salt water-culture series containing chlorate showed essentially the same results as the soils.

Absorption experiments with squash plants in high- and low-nitrate aerated culture solutions indicate that little chlorate is absorbed from high-nitrate cultures, whereas chlorate is absorbed in greater quantity and accumulated in the xylem sap in low-nitrate cultures.

Chlorate absorption seems to be a function of root activity and of the concentration and physical and chemical properties of the anions in the culture medium. Under low-nitrate conditions the plant will take up chlorate to its own detriment against a concentration gradient.

Seasonal changes in the response of plants to chlorate application through the soil may be explained on the basis of nitrate concentrations as affected by leaching, temperature, absorption by plants, and nitrification.

Since in most soils fertility, as denoted by the production of crops and roadside plants, is determined by nitrate concentration, plant growth may be used in predicting response to chlorate applications. In highly fertile soils costly experimentation with chlorates may be avoided and a program using carbon bisulphide or cultural or cropping practices substituted.

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SOIL STUDIES ON THE CAUSES OF THE BROWN ROOT ROT OF TOBACCO[1]

By E. D. MATTHEWS and C. A. RENEGER, *formerly graduate assistants*, and R. P. THOMAS, *professor of soil technology, Agronomy Department, Maryland Agricultural Experiment Station*

INTRODUCTION

Brown root rot of tobacco has occurred in nearly every area where tobacco (*Nicotiana tabacum* L.) is extensively grown. In Maryland the loss by this disease has varied. In some years the damage has been great, and in others very little has been found. This variation in the intensity of the disease suggested that a study of the environmental conditions might be helpful. This paper reports a study of some of the chemical and biological factors found in soil infected with brown root rot.

HISTORICAL REVIEW

Many causal agencies have been suggested for tobacco brown root rot (*4, 5, 8, 9, 10, 21*).[2] These agencies can be classified roughly as parasitic organisms, toxic substances in the soil, and nutritional disturbances. The following facts suggest that the disease is associated with an organism: The disease can be transferred to some healthy soils by the addition of the infected roots; sterilization destroys the disease; partial sterilization reduces the infection temporarily; heating a soil, so long as the soil micro-organisms are not destroyed, increases the amount of the disease; and the dilution of the diseased soil with healthy soil decreases the infection. However, there were many experimental facts observed in the study of the disease and related field conditions which could not be directly associated with an organism. Attempts to isolate an organism that would reproduce the disease were unsuccessful. Air-drying the infected soil decreased or destroyed the ability of the soil to reproduce the brown root rot. Continuously cropping soil to tobacco caused the disease to disappear.

Since the brown root rot was much worse when certain crops, as timothy (*Phleum pratense* L.) preceded a tobacco crop, it was suggested that the disease resulted from toxic substances left in the soil by some of the previous crops. Aluminum was thought to be one of those important toxic substances in the soil until it was found that application of lime decreased the amount of soluble aluminum and increased the brown root rot. The disease was found to be associated with high organic-matter content and low nitrate content of soils. The apparent control of the brown root rot by the use of large amounts of fertilizer and the ability to bring it back into the soil by applying large amounts of organic materials were also demonstrated. All of these data indicate that the disease is the result of some unbalanced condi-

[1] Received for publication July 22, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 683.

tion of the soil. This unnatural disturbance may be purely nutritional although it seems more logical that it may be caused by undesirable soil flora.

EXPERIMENTAL PROCEDURE

A study was made of the soil conditions in some badly infected tobacco soils to determine some of the fertility and biological factors associated with brown root rot. These studies consisted of determining the nitrate nitrogen, water-soluble phosphorus, total carbon, and cellulose content. The observations made in the field at each sampling suggested some unnatural activity in the soil flora. In order to test these field observations, many of the soils and infected roots were plated out in different media. A fungus was isolated that would reproduce the disease in healthy soil, when the proper conditions were present. Cultures were made of the fungus to determine its preference in nitrogen, energy, and reaction. Greenhouse tests were conducted to determine the soil conditions required to cause this organism to produce browning of the tobacco roots.

FIELD PLOTS

The field work was conducted on several of the rotation plots at the Marlboro tobacco station and the experimental plots at Cheltenham and Leonardtown, Md. The soil of these plots was sampled in May, June, and July. Nitrate nitrogen, water-soluble phosphorus, total organic matter, and cellulose determinations were made on these samples. Observations on the percent of infection of the brown root rot were made throughout the growing season of the tobacco. There was considerable variation in the nitrate-nitrogen content, especially when different crops had been turned under and different kinds and amounts of fertilizer had been added. The amount of water-soluble phosphorus varied greatly, the most noticeable variation being the effect of fertilizer. The total carbon, and the cellulose content fluctuated widely. There was as much variation between the duplicate plots as between plots receiving different treatments. The results of this study were more or less negative in that it was impossible to associate the presence or absence of the disease with the amount of nitrate nitrogen, water-soluble phosphorus, total carbon, or cellulose. There was a possibility that the dry seasons of 1929 and 1930, when the soil samples were obtained, prevented the proper development of the disease in the field. It is interesting to note that beginning in 1929 there were 5 years in which it was dry during the late spring and early summer. During this time much of the brown root rot of tobacco disappeared in Maryland.

PLATING OUT OF ROOTS

The observations in the field suggested, as they had previously to many other investigators, that the disease was associated with some organism. Accordingly, many samples of infected roots and soils which produced the disease were plated out in several culture media. A large number of fungi were obtained, but the majority of them were the usual rapid-growing fungi secured on soil plates. By accident some of the Czapek's agar plates were left in the incubator much longer than usual before discarding. On examining these plates an entirely different organism was noted.

Pure cultures of this organism were grown and used to inoculate soils and sands that were growing healthy tobacco. In the case of soil that normally produced the disease in the field, the inoculation with pure cultures of this fungus produced the disease even when the soil had been sterilized. The inoculation of healthy soil (which did not normally produce the disease in the field) or quartz sand with cultures of the fungus seldom produced any lesions of brown rot on the tobacco roots. However, if these soils or sand contained considerable organic matter the inoculation of the cultured fungus caused typical brown root rot lesion on the tobacco. These results indicated that this organism is associated in some manner with the brown root rot of tobacco.

DESCRIPTION OF ISOLATED ORGANISM

The fungus was early recognized as being either a *Sclerotium* or a *Rhizoctonia*. John A. Stevenson, senior mycologist of the Bureau of Plant Industry, identified it as *Sclerotium bataticola*, Taub. From the different cultures it was observed that the form-generic names *Sclerotium* and *Rhizoctonia* could quite correctly be used interchangeably for the designation of the sterile stage of this fungus, depending upon the character of the individual colony of the fungus under observation. However, with a few exceptions the fungus was observed to be of the form genus *Sclerotium* and corresponded morphologically to the original description by Taubenhaus (*20*, p. *164*).

Sclerotia jet black, very minute; exterior smooth, made up of anastomized black hyphae; interior light to dark brown, made up of free, thick-walled cortical, hyphal cells; sclerotia vary much in shape, spherical, oval, oblong, elliptical, curved or even forked, varying in size from $25\times22.4\mu$ to $152\times32\mu$, abundant throughout the entire root of the host.

Many workers in other countries described a similar fungus and named it *Rhizoctonia*. In 1925 Briton-Jones (*1*) compared cultures of this *Rhizoctonia* with a culture of *Sclerotium bataticola* received from Taubenhaus and found that they were the same. Therefore, he suggested that the name *Rhizoctonia bataticola* (Taub.) Butler be applied to this sclerotial fungus. This synonym is widely used in the literature outside the United States. Some of these writers designate it *Rhizoctonia* (*Sclerotium*) *bataticola*. A pycnidial stage of a fungus of this type has been referred to as *Macrophomina phaseoli* Ashby. The fungus isolated in this work from soil and tobacco roots was cultured on many media, and no other form developed. Therefore it seems best to call it *Sclerotium bataticola*.

RESPONSE OF ORGANISM TO DIFFERENT ENVIRONMENTAL CONDITIONS

From the observations reported by many of the previous investigators it was concluded that if the brown root rot of tobacco is produced by a parasite, the organism is different from the usual parasite. In order to determine whether *Sclerotium bataticola* had these abnormal characteristics, pure cultures of the organism were subjected to many different environmental conditions. Some of the fungus cultures were dried in the air and in desiccators both within and without the soil, and the effect upon viability was noted. Cultures were grown on many types of media. The relation between the hydrogen-ion concentration and the growth of the organism was

studied. The ability of this fungus to utilize various kinds of carbohydrates and different forms of nitrogen was also investigated.

The effect of drying upon the viability of *Sclerotium bataticola* was determined by using diseased soil, healthy soil, and sand. Each sample of soil and of sand was divided into two portions. One lot was for air-drying and the other for incubation under moist conditions. The healthy soil and sand were sterilized and inoculated with cultures of the fungus. The portion of each soil and the sand saved for incubation were made up to optimum moisture and placed in an incubator in the beginning to prevent drying. The remaining parts of the soils and the sand were spread out in a thin layer and air-dried for 4 days. Then each sample was brought to approximate optimum moisture and incubated at 28° C. for 3 weeks. At the beginning of the experiment all soils were plated out on Czapek's solution agar. The fungus was cultured only from the undried portions of the diseased and the healthy inoculated soils and sand. After 2 and 3 weeks of incubation these soils were plated out again on Czapek's agar. With one exception no *S. bataticola* was obtained from any of the soils or sand which had been dried. This exception was thought to be the result of insufficient drying.

Further tests on the effect of drying on the fungus were made. Bits of mycelia were removed from 3-day-old cultures grown in test tubes of sand which had been saturated with Czapek's solution. Each piece of mycelium was air-dried at room temperature for various periods of time and then placed on the surface of Czapek's solution agar in Petri dishes. Ten minutes was sufficient to destroy the viability of the young mycelia. Often 3 minutes of drying prevented growth. The sclerotia which started forming in the test tubes 4 days after inoculation were removed on the 10th day and dried for various periods under different conditions. Some were dried at room conditions, and some in a desiccator over concentrated sulphuric acid. After drying, the sclerotia were placed on Czapek's solution agar and incubated at 28° C. The drying at room temperature rapidly destroyed the viability of the sclerotia; only 1 out of 13 germinated after exposure for 1 hour, and only 1 out of 14 after exposure for 1½ hours. The drying in the desiccator killed the sclerotia much more rapidly. Some of these drying experiments were repeated, mycelia and sclerotia approximately 30 days old being used. The older sclerotia were not completely destroyed until dried for 70 hours or more in a desiccator. This behavior of the organism agreed closely with the results obtained when brown root rot-producing soil was aid-dried.

INOCULATION TESTS

Since previous investigators reported varying degress of infection following different crops it was thought that perhaps the residues in the soil might stimulate this fungus differently. Accordingly, the organism was cultured on several media (*6*), including Czapek's, Waksman's sweetpotato, malt extract, cellulose peptone, ammonia sulphate, and cellulose agars. Except on the Waksman's agar the fungus grew well on all of these media. The colony diameters on the plates were slightly larger on the cellulose peptone agar than on the ammonium sulphate-cellulose agar. This suggested that perhaps the form of nitrogen required by this organism might be very specific.

In order to check this, media containing different sources of nitrogen were made up as suggested by Norman (*13*). Peptone, sodium nitrate, ammonium sulphate, and sodium nitrite were used as sources of nitrogen. The reactions of all the media were adjusted to pH 5.5. Ten plates were poured of each medium, and young sclerotia from Czapek's solution agar were added to each. The results, as presented

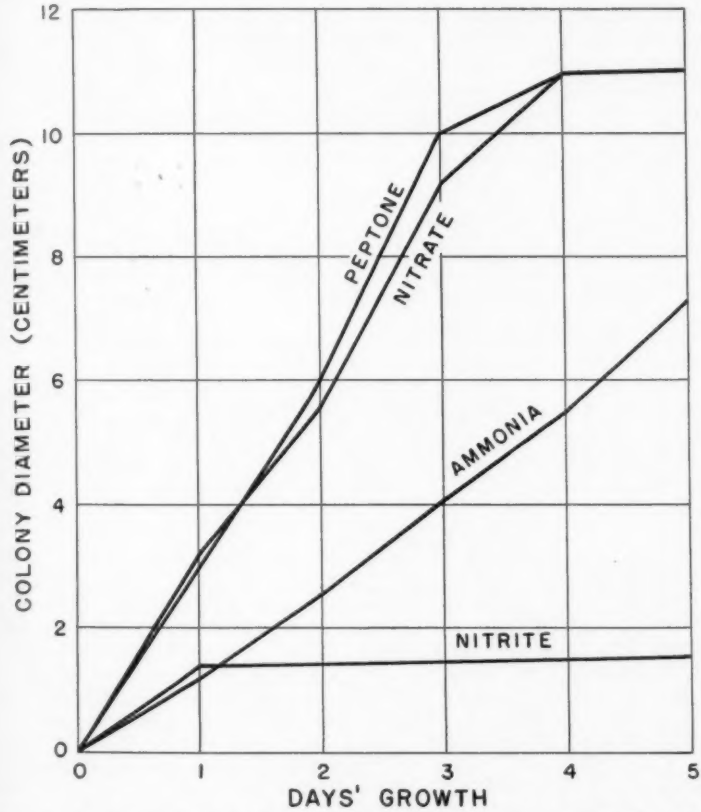

FIGURE 1.—Growth of *Sclerotium bataticola* Taub., as measured by colony diameters, on agar media in which the nitrogen was derived from different sources.

in figure 1, indicated that *Sclerotium bataticola* seemed to prefer peptone nitrogen although the nitrate nitrogen produced almost as much growth.

In the tests on the different media, growth was considerably inhibited on Waksman's agar. Since this medium was the only one which had been adjusted to a pH value as low as 4, it was thought that reaction might have considerable effect on the growth of *Sclerotium bataticola*. In order to test this, various portions of Waksman's medium were

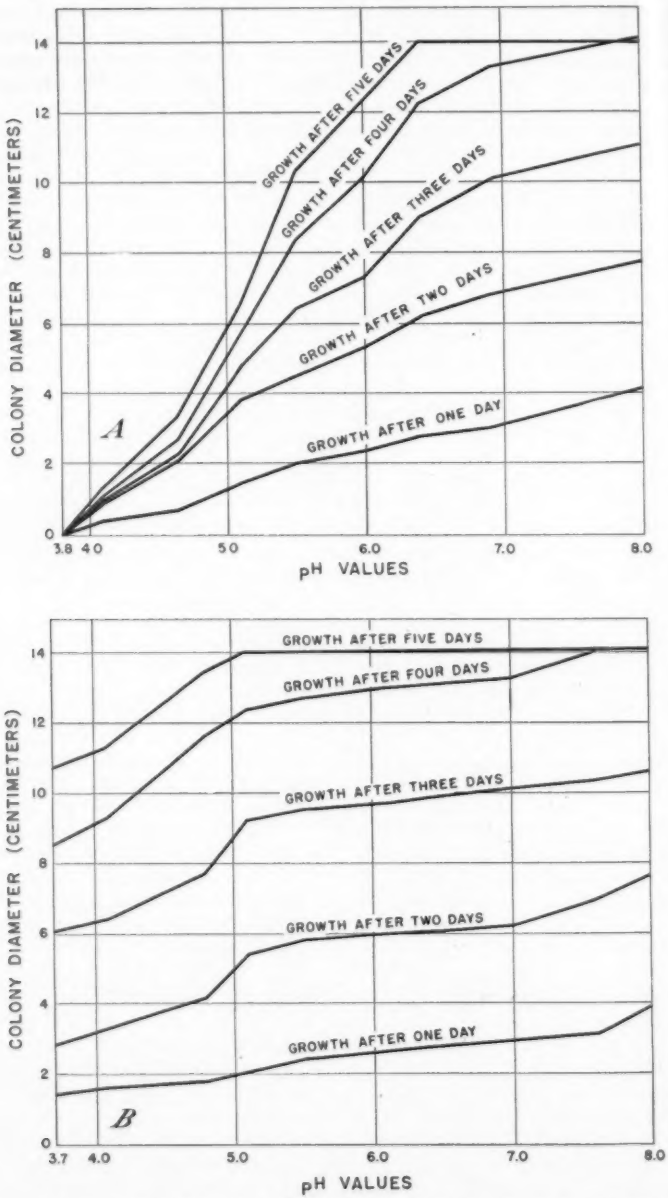

FIGURE 2.—Growth of *Sclerotium bataticola* Taub., as measured by colony diameters, on Waksman's (*A*) and on Czapek's (*B*) agar at different pH values.

made up with pH values varying from 3.8 to 7.9. After the plates were inoculated, they were incubated at 28° C and the colony diameters measured every 24 hours for 5 days. As can be seen in figure 2, *A*, there was considerable difference in the growth. The low pH values retarded the growth very noticeably. The higher the pH value the greater the growth of the organism. Although the optimum reaction was not determined, the results indicated that it would be on the alkaline side of neutrality. This experiment was repeated by using the poorly buffered Czapek's solution agar (*11*). The results as given in figure 2, *B*, are very much different. In the Czapek medium growth was retarded only slightly at the low pH value, although the colony diameter increased with an increase in pH value. It is believed that with this medium the reaction changed quite rapidly under the influence of the growing organisms and the early retardation was quickly overcome.

In some of the previous experiments it was noted that the source of carbohydrates seemed to affect the growth of the organism. Hence the source of carbohydrates was varied; peptone was used as the source of nitrogen. The carbohydrates used were sucrose, lactose, mannite, glucose, potato starch, soluble starch, and cellulose. Each was applied at the rate of 15 g per liter except the cellulose, which was added as precipitated cellulose at the rate of 5 g per liter. The reaction was adjusted to pH 5.6. The results of this experiment showed no significant differences in colony diameter, appearance, growth, or sclerotial forms for the different sources of carbohydrate. This same experiment was repeated by using sodium nitrate instead of peptone as the source of nitrogen. The results were very similar to those of the preceding test, although the peptone media might have had slightly more dense colonies and produced a greater number of sclerotia. From these results it was concluded that *Sclerotium bataticola* is able to utilize several different kinds of carbohydrate equally well.

The apparent ease with which the organism decomposed cellulose led to further test of this ability. Sterilized strips of filter paper in Omeliansky's solution agar (*6*) were readily destroyed by the fungus. Finely ground crude paper was mixed with white sand, sterilized, then moistened with sterilized Czapek's solution, and inoculated with the organism. The paper was rapidly decomposed. However, when an attempt was made to culture the organism on lignin prepared from crude paper, no growth was obtained.

It was apparent from the behavior of *Sclerotium bataticola* in the pure-culture experiments that it could grow well under the conditions observed in the field where the disease were reported. In order to test this hypothesis, several pot tests with tobacco and tomatoes were made. In the first, disease-free or healthy soils were used. The soil treatments, are given in table 1. The organic materials were applied at the rate of 3 tons per acre. The soil was sterilized just before the addition of the inoculant. Young tobacco plants, grown in sterilized quartz sand and nutrient solution, were transplanted to these pots. After a few weeks' growth the plants were removed and the roots examined for the presence of brown root rot lesions. No lesions appeared except where both the filter paper and the inoculant were

added. The pots receiving the ground timothy roots made a much better growth. Evidently the sterilizing of this material made considerably more plant food available. This experiment was repeated by using finely ground paper toweling at the rate of 3 tons per acre, with tomatoes as the index crop. Although the roots of the tomato plants after 5 weeks' growth were discolored, they did not have the typical brown root rot lesion.

TABLE 1.—*The influence of timothy residues, cellulose, and the presence of Sclerotium bataticola on the growth of tobacco in pots, and the occurrence of brown root rot in healthy soil*

Treatment [1]	Symptoms of brown root rot	Average dry weight of plants	Relative dry weights
		Grams	
Control	Absent	6.48	100
Inoculated	do	6.60	102
Timothy roots; uninoculated	do	10.52	162
Timothy roots; inoculated	do	3.65	56
Filter paper; uninoculated	do	.45	7
Filter paper; inoculated	Present	.40	6

[1] Organic materials applied at rate of 3 tons per acre. Soils sterilized just before inoculation.

Since brown root rot apparently occurs in the field only on certain soils and under certain conditions, it was thought that perhaps none of these experiments duplicated the exact conditions for the disease. Accordingly, another inoculation experiment was made. This time soil which was known to produce the disease in the field was used. The soil treatments were control, steam steilized, cellulose added, and inoculated, in various combinations, as reported in the following tabulation.

Soil treatment:	*Severity of brown root rot*
Control, no treatment	Moderate.
Steam sterilized	None.
Cellulose added	Moderate.
Cellulose added, sterilized	None.
Inoculated	Moderate.
Sterilized, inoculated	Do.
Cellulose added, inoculated	Moderate to severe.
Cellulose added, sterilized, inoculated	Do.

Cellulose, as finely ground crude paper, was added at the rate of 3 tons per acre. The soils were sterilized for 2 hours after the cellulose had been added and before they were inoculated with cultures of *Sclerotium bataticola*. Tobacco seedlings were grown in these soils for 3 weeks. Brown rot appeared on all the tobacco roots except those in the soils which were sterilized and not inoculated.

The observations of the culture habits of *Sclerotium bataticola* indicated that this organism grew under conditions similar to those reported for brown root rot in the field, also that it could be controlled in very much the same way as in the field. This agreement between the cultural habits and field observations, together with the production of typical root rot lesions in the greenhouse, shows that the disease known as brown root rot of tobacco is produced by the fungus *S. bataticola*.

DISCUSSION

RESPONSE OF ORGANISM TO ENVIRONMENTAL CONDITIONS SIMILAR TO THOSE IN THE FIELD

It is believed that the results of these studies have pointed out fallacies in the previous explanations of causes of this disease. The killing of the fungus *Sclerotium bataticola* by drying, both in the soil and in cultures, agrees with the previous experiments reported upon air-drying of the soil. The authors' inability to plate out the fungus from desiccated soil was also similar to that reported by early workers. The apparent need of this fungus for energy material offers an explanation of the decrease in infection when tobacco is grown continuously. The stimulating effect of lime on the disease in the field correlated well with the tests made upon the effect of hydrogen-ion concentration on the colony diameter of cultures of this fungus. Apparently this fungus is parasitic only under certain environmental conditions. This is in agreement with the results reported by Ciferri (*3*) who showed that any conditions favorable to the luxuriant growth of a *Rhizoctonia* in the soil also favored the parasitism of this organism on the roots of the plants. These observations show that *S. bataticola* could be more parasitic under certain conditions.

Since available organic matter and nitrogen stimulate the activity of this organism, it is believed that this explains the low amount of nitrates in the soil at the time of the greatest activity of the fungus. This belief is further supported by the fact that the soils infected with brown root rot contain considerable plant residues such as cellulose, which has been shown to stimulate the growth of the organism. In the decomposition of these organic materials the fungus probably consumes all of the readily available nitrogen in the soil, and in its search for nitrogen it invades the tobacco roots. The roots of tobacco were found to be very high in nitrogen, especially the nitrate form. This hypothesis, previously suggested by Thomas (*21*), is substantiated by the results obtained when the addition of excessive amounts of cellulosic material nearly always increased the amount of root lesions on tobacco grown in inoculated soils.

EXPLANATION OF FACULTATIVE PARASITISM

The literature indicates further that conditions known to be favorable to brown root rot are similar to, if not identical with, the conditions which produce facultative parasitism of the common endophytic fungi, especially the "*Rhizoctonia*" group. Peyronel (*15*) found that an excess of organic material in the surface layers of the soil favored a luxuriant development of "*Rhizoctonia*." Extreme moisture conditions were also favorable. This was substantiated by Ciferri (*3*) who showed that any factor which limited the rapid formation of any new roots, or destroyed the equilibrium between root formation and root loss, caused endophytic fungi to become actively parasitic. This was especially true of "*Rhizoctonia*" and similar fungi.

Many of the early workers reported that there seemed to be an association between the root lesions and mycorrhizae in the soil. Ciferri (*3*) indicated this quite strongly in his work with sugar cane. He reported that a "*Rhizoctonia*" was the immediate cause of the root disease and existed endophytically in apparently healthy roots. They became actively parasitic only under certain conditions unfavorable

to the plant. He cited Peyronel (*15*) to substantiate these observations. Hopkins (*7*) and Park (*14*) reported that *Sclerotium bataticola* could cause root disease of a number of plants although the fungus commonly existed in mycorrhizal relationship with plant roots. Such a relationship did not seem obligatory. Steinmann (*19*) concluded from his observations that "*Rhizoctonia*" *bataticola* was a common mycorrhizal fungus which under certain conditions became parasitic. He definitely proved this to be the case in the root disease of tea.

A mycelial development within the diseased portion of the tobacco roots was reported by Johnson, Slagg, and Merwin (*10*) early in the study of this disease. The presence of *Rhizoctonia* was also noted by these workers. The stained sections of the roots did not always indicate the presence of an organism. This is in agreement with the work of Steinmann (*19*) who found that hyphae of "*Rhizoctonia*" *bactaticola* were hardly distinguishable in the cells and later apparently disappeared with the advance of the disease on tree roots. Many workers (*2, 12, 14, 18, 19, 22, 23*) have given a large number of host plants for *Sclerotium bataticola*. It is interesting to note that the list of plants susceptible to brown root rot (*10*) contains many plants which other workers have shown to be the host plants. Tobacco has never been listed as a host plant of *S. bataticola* in this country, although it has been in other parts of the world (*2, 16, 17*). Also, this fungus has been associated with the production of other root diseases.

From the results of this study and the observations reported by other workers, it is concluded that *Sclerotium bataticola* can cause brown root rot of tobacco. It has not been proven that this is the only fungus capable of producing the disease. Other organisms of similar morphological and physiological characteristics might be associated with the cause of the disease, although none were isolated in this study. There is a possibility that *S. bataticola* is only indirectly the cause of the disease. Regardless of how this organism produces the root rot infection, it should be remembered that the relation of the chemical, physical, biological, and environmental conditions of the soil to both the tobacco plants and soil flora is very important.

SUMMARY

A study was made of the chemical and biological soil factors which might be associated with the occurrence of brown root rot in tobacco. The nitrate-nitrogen, water-scluble phosphorus, cellulose, and carbon content of the soils were determined on field plots planted to tobacco. Many of the infected tobacco roots and soils were plated out to isolate a fungus which might be associated with the disease. From these cultures an organism (*Sclerotium bataticola*) was obtained which was found to be associated with the disease. The cultural habits of this organism in the laboratory were then studied to see whether its growth habits were similar to those in the field. Finally inoculation experiments were conducted in an attempt to reproduce the disease in the greenhouse. A fungus (*S. bataticola*) was isolated from the infected tobacco roots and from the soil in which these roots grew. From the results of these studies the following conclusions may be drawn: This fungus is readily destroyed by air-drying, it is a cellulose-decomposing organism, it can readily utilize either nitrate or organic forms of nitrogen, and it grows better in neutral to alkaline media.

These characteristics are apparently in accordance with the conditions in the field which influenced the occurrence and severity of the disease. In inoculation experiments in the greenhouse under controlled conditions it was found that *S. bataticola* produced the brown root rot of tobacco.

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A FOLIAR DIAGNOSIS STUDY OF THE INFLUENCE OF CALCIUM FROM TWO SOURCES, LIME AND SUPERPHOSPHATE[1]

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INTRODUCTION

In the preceding paper of this series[2] the course of nutrition of maize plants (*Zea mays* L.) growing on eight plots of tier 1 of the Jordan fertility plots of this experiment station was described.

The treatments of these particular plots consisted of a single element, combinations of two elements, and a complete fertilizer containing all three elements, nitrogen, phosphorus, and potassium. The carrier of nitrogen was dried blood; of phosphorus, the superphosphate of commerce; and of potassium, the chloride or muriate.

Plot No. 1 (check) has never received any fertilizer or lime. But another plot (No. 23), not considered in the previous paper,[2] has received calcium in the form of lime, without any commercial fertilizer, every 4 years at the rate of 500 pounds per application to the ⅛-acre plot. Four of the plots, P (No. 3), NP (No. 5), PK (No. 7), and NPK (No. 9), received calcium in the form of ground bone black until 1917. Since then they have received superphosphate at such a rate as to provide 48 pounds of P_2O_5 to the acre, applied to the corn and wheat. Plot No. 23 accordingly received 3,000 pounds of calcium in the form of lime (CaO) up to 1936, and plots Nos. 3, 5, 7, and 9 received during the same 56-year period (1881–1936) approximately 400 pounds of calcium present in the superphosphate and ground bone black.

The object of the present investigation was to compare the foliar diagnosis of corn plants growing on a plot receiving lime only with that of corn plants on a check plot receiving no lime, and to determine the relationship of the foliar diagnosis of plants growing on plots receiving calcium in the form of superphosphate to that of plants on a plot receiving lime only. The results are reported herein.

EXPERIMENTAL PROCEDURE

The arrangement of the experimental plots has been described previously.[2]

The sampling of the leaves was carried out in the manner described in detail in an earlier paper.[3] The third leaf from the base of the stalk was taken periodically during the season from all of the plants lengthwise of each plot. Successive rows were sampled on each of the sampling dates indicated in table 1.

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PRESENTATION AND DISCUSSION OF RESULTS

The composition of the third leaf expressed as percentages of N, P_2O_5, K_2O, CaO, and MgO in the dried foliage and the composition of the NPK unit [2] and yields of grain and stover of plants from plots No. 1 (check) and No. 23 (lime), are listed in table 1 and shown graphically in figures 1 to 3.

For purposes of orientation the derivation of the NPK unit represents the equilibrium between N, P_2O_5, and K_2O in the chosen leaf at the moment of sampling and is derived by converting the percentage composition for N, P_2O_5, and K_2O into milligram-equivalent values and then determining the proportion each of these bears to the milligram-equivalent total.[3]

COMPARISON OF THE FOLIAR DIAGNOSIS OF PLANTS ON THE LIME PLOT (NO. 23) WITH THAT OF PLANTS ON THE CHECK PLOT NO. 1

INDICATIONS GIVEN BY THE GRAPHS SHOWING THE PERCENTAGE OF N, P_2O_5, K_2O, CaO, AND MgO AS ORDINATES AND DATES OF SAMPLING AS ABSCISSAE

TABLE 1.—*Percentage composition expressed in terms of the dried foliage together with the composition of the NPK unit and yield of plots No. 1 (check) and No. 23 (lime)*

Date and plot	N	P_2O_5	K_2O	N+P_2O_5+K_2O (s)	CaO	MgO	Composition of the NPK unit (relative proportions of N, P_2O_5, K_2O)			Yield per plot	
							N	P_2O_5	K_2O	Grain	Stover
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Pounds*	*Pounds*
Check No. 1:											
July 6	2.960	0.307	2.387	5.654	2.308	0.934	76.804	4.719	18.477	165.4	117.1
July 21	2.210	.305	2.515	5.030	2.087	.966	70.361	5.753	23.886		
Aug. 8	1.800	.230	1.841	3.871	1.605	1.039	72.421	5.482	22.097		
Aug. 25	2.010	.316	1.608	3.936	1.999	1.216	75.087	6.993	17.920		
Lime No. 23:											
July 6	3.260	.582	.872	4.714	4.144	1.376	84.348	8.921	6.731	452.2	222.1
July 21	2.710	.568	.910	4.188	4.233	1.231	81.676	10.142	8.182		
Aug. 8	2.410	.474	.910	3.794	3.851	1.122	81.356	9.480	9.164		
Aug. 25	2.340	.524	.891	3.755	3.983	1.014	80.241	10.645	9.114		

In the following discussion it is necessary to refer continually to the content of, or the graph for, nitrogen, phosphoric acid, potash, lime, or magnesia of the selected leaf (third from the base) of plants growing on a particular plot. To avoid monotonous repetition and a cumbersome structure, the plot treatment or its symbol (check, N, P, lime, etc.), is used to refer to leaves from plants growing on the plot indicated.

NITROGEN

The graphs for nitrogen are unlike both in form and position; the graph of lime (No. 23) is higher than that of the check (No. 1) throughout the whole period. At the third sampling the difference is as great as 0.61 percent.

PHOSPHORIC ACID

The graphs for phosphoric acid are similar in form but not in position. The graph of lime (No. 23) is much above that of the check (No. 1) throughout the whole period, an indication that inhibition of

[2] THOMAS, WALTER. See footnote 3, page 685.
[3] THOMAS, WALTER, AND MACK, WARREN B. See footnote 2, page 685.

the absorption of P_2O_5 resulting from the medium (soil) has been removed by liming. The mechanism of this phenomenon has been described.[4]

POTASH

The graphs of potash are unlike both in form and position. The graph of lime (No. 23) is always much below that of the check (No. 1). During the early period the K_2O content of the selected leaf from plants on plot receiving lime (No. 23) is less than one-half that of the

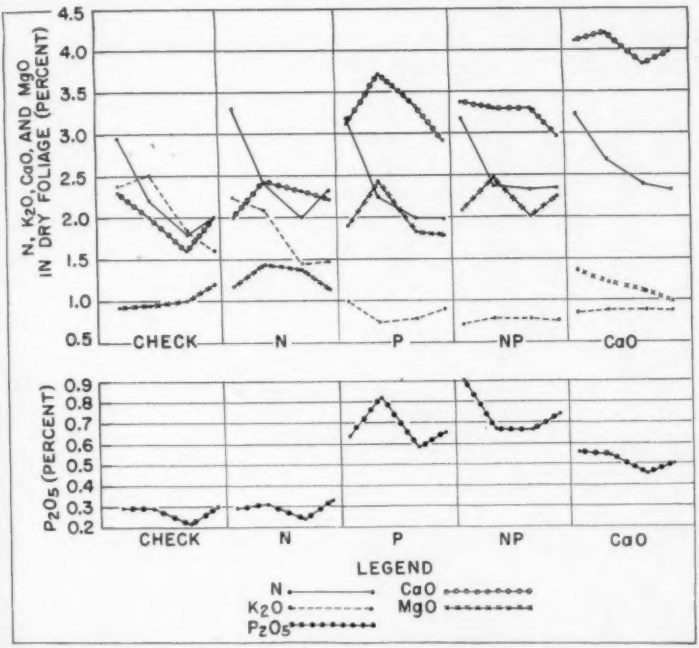

FIGURE 1.—The nitrogen, phosphoric acid, potash, lime, and magnesia content of the third leaf plotted as ordinates, and dates of sampling as abscissae. (The dates of sampling are July 6, July 21, August 8, and August 25, and are indicated by the four dots in each curve.)

selected leaf from plants on check plot (No. 1). Lime, consequently, has reduced the absorption of potassium by the plant, and the seat of this inhibition lies in the soil and not in the plant.

LIME

The graphs of lime differ in form and position. The graph of lime (No. 23) is very much higher than that of the check (No. 1) throughout the whole period, a reflection of the fact that certain functions of potassium can be performed by calcium when the former is deficient.[5]

[4] THOMAS, WALTER. PROPERTIES OF THE HYDROXYL GROUPS OF CLAY AS A BASIS FOR CHARACTERIZING A MINERAL SOIL. Soil Sci. 42: 243-259, illus. 1936.

[5] THOMAS, WALTER. MATHEMATICAL EXPRESSION OF EQUILIBRIUM BETWEEN LIME, MAGNESIA, AND POTASH IN PLANTS. Science 88: 222-223, illus. 1938.

MAGNESIUM

The graph of the check (No. 1) rises progressively, whereas that of lime (No. 23) falls progressively with the advancing age of the leaf. Such relative differences between import of Mg into the leaf and export from it are an indication that the demand by the plants on the former plot is always less, and on the latter always greater than the supply.

INDICATIONS GIVEN BY THE GRAPHS SHOWING THE NPK UNITS IN TRILINEAR COORDINATES

The composition of the NPK units at the various sampling dates is given in table 1. The mean intensity of nutrition and the mean

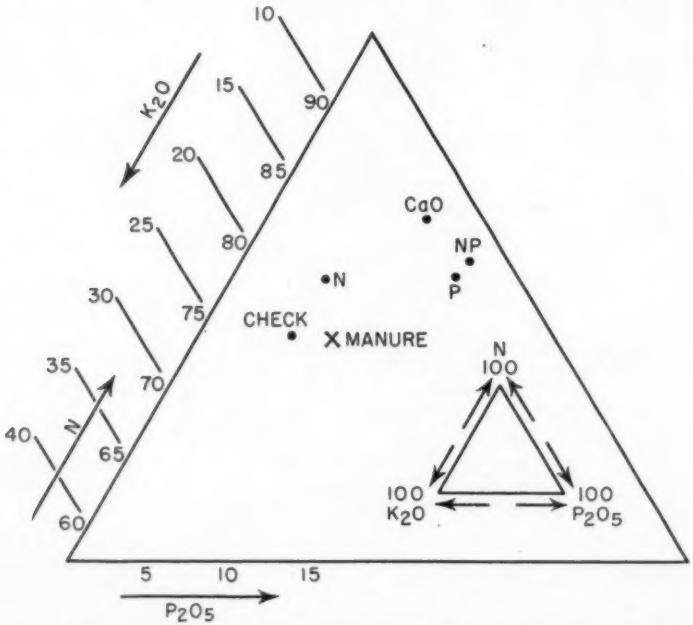

FIGURE 2.—Positions of the mean NPK units of the third leaf from the check plot, and from plots receiving dried blood, superphosphate, and lime, compared with that of the optimum (manure+CaO).

NPK units are given in table 2, and the latter are shown graphically in figure 2.

Liming reduced the intensity of nutrition by its effect in reducing the absorption of potassium. The effect of liming on the N—P_2O_5—K_2O equilibrium was to increase the nitrogen of the NPK unit at the the expense, principally, of the potassium.

In figure 2 the position of the mean NPK unit of the treatment resulting in the highest yields of the plots on tier 1, namely manure+CaO, is marked by a cross (×). Check (No. 1) has a lower intensity than has manure+CaO (No. 22), with insufficient P_2O_5 and

a slight excess of K_2O in the NPK unit. Lime alone resulted in reducing the intensity below that of the check (No. 1). The notable effect of liming is on the composition of the NPK unit, the K_2O of which is reduced from a value of 20.595 in the check plot (No. 1) to 8.298 in the lime plot (No. 23), and the P_2O_5 increased by more than 70 percent.

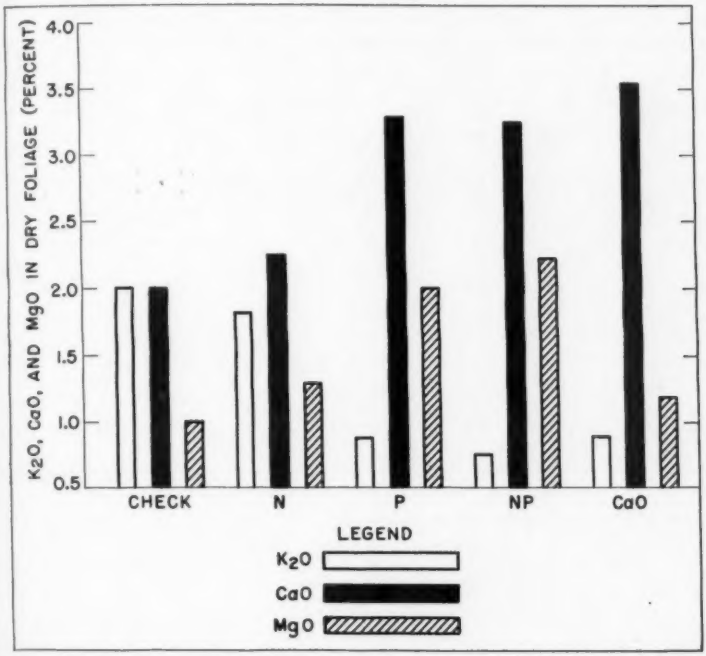

FIGURE 3.—The mean content of potash, lime, and magnesia, respectively, of the third leaf of plants from the check plot, and from the plots receiving dried blood, superphosphate, and lime.

TABLE 2.—*The mean intensity of nutrition, the mean NPK unit and yield of plot No. 1 (check) and plot No. 23 (lime), and plot No. 22 (manure)*

Plot No.	Treatment	Intensity of nutrition	Composition of the mean NPK unit [1]			Yield of grain per plot
			N	P_2O_5	K_2O	
			Percent	*Percent*	*Percent*	*Pounds*
1	Nothing	4.62	73.668	5.737	20.595	165.4
23	Lime	4.11	81.905	9.797	8.298	452.2
22	Manure	5.88	73.376	8.234	18.390	770.8

[1] The values of the mean intensity of nutrition and of the mean NPK unit for the respective treatments are the mean of the values for the intensities and NPK units, respectively, at the four dates of sampling recorded in column 5 and in columns 8, 9, and 10 of table 1.

COMPARISON OF THE FOLIAR DIAGNOSIS OF PLANTS GROWING ON PLOTS P (NO. 3), NP (NO. 5) AND LIME (NO. 23) WITH THAT OF CHECK PLOTS (NO. 1) AND N (NO. 2)

INDICATIONS GIVEN BY THE GRAPHS SHOWING THE PERCENTAGE OF N, P_2O_5, K_2O, CaO, AND MgO AS ORDINATES AND DATES OF SAMPLING AS ABSCISSAE

The K_2O graphs of P (No. 3), NP (No. 5), and lime (No. 23) are characterized by relatively small changes with advancing age of the leaf. The K_2O graph for lime (No. 23) differs but little either in form or position from the graphs for K_2O of P (No. 3) and of NP (No. 5), which are the lowest of any treatments in tier 1.[6] The comparisons are brought out better in table 3 and figure 3, which show the mean percentage composition with respect to K_2O, CaO, and MgO, respectively, at the four sampling dates.

TABLE 3.—*Comparison of the foliar diagnosis and yield of plants on the P plot (No. 3), NP plot (No. 5), and lime plot (No. 23) with check plot (No. 1) and N plot (No. 2)*

Plot No.	Treatment	K_2O	CaO	MgO	Yield of grain per plot
		Percent	*Percent*	*Percent*	*Pounds*
3	P	0.87	3.29	2.00	371.2
5	NP	.75	3.26	2.23	376.7
1	Nothing	2.09	2.00	1.03	165.4
2	N	1.82	2.26	1.29	265.6
23	CaO	.89	3.55	1.18	452.2

The addition of phosphate only and also of phosphate combined with nitrogen (NP) has resulted in a reduction of the potash content of the leaf by one-third of the values found in the check (No. 1) and also in N (No. 2). Liming has produced effects quantitatively similar. The conclusion is that the element common to both treatments—calcium—is the causal factor.

LIME AND MAGNESIA

In a preceding paper,[6] it was shown that the CaO graphs of P (No. 3) and also of NP (No. 5) were much higher than that of any of the other treatments without lime, viz. (check, N, K, NK, PK, NPK), and that throughout the whole period, the CaO content of the leaf from plots receiving superphosphate without potash (P, NP), were only slightly lower than the CaO content of the leaf from the limed plot, whereas the MgO content was actually greater. McIntire[7] stated many years ago that—

> the application of phosphorus in the form of dissolved bone black (used as the carrier of phosphorus prior to 1890) carries at least one-half as much $CaSO_4$ as is applied on plat 33 (gypsum only), and it is possible that part of the increase upon the phosphorus treated plats is due to the lime or sulphur in the gypsum contained in the dissolved bone material * * *.

But the element sulphur is not present in the lime added to plot No. 23, which fact eliminates this factor from consideration here.

[6] THOMAS, WALTER, and MACK, WARREN. B. See footnote 2.

[7] MCINTIRE, W. H. RESULTS OF THIRTY YEARS OF LIMING. Pa. Agr. Expt. Sta. Ann. Rept. 1911-12: 64-75. 1913. See p. 71.

The writers indicated in the previous paper[8] that the CaO and MgO graphs of plots PK (No. 7) and NPK (No. 9) which received both superphosphate and potash were below those of plots P (No. 3) and NP (No. 5) which received superphosphate without potash. Their findings give additional confirmation of the reciprocal effects of potassium and calcium and of the fact that the seat of this influence is located in the soil and not in the plant.

Indications Given by the Graphs Showing the NPK Units Plotted in Trilinear Coordinates

The mean intensities and the mean NPK units of the third leaf are shown in table 4 and graphically in figure 2. The calcium of superphosphate has had a depressing effect on the intensity of nutrition (table 4, column 3) just as lime has had.

The mean NPK units (fig. 2) of plots P (No. 3), NP (No. 5), and lime (No. 23) are displaced relatively higher up and farther toward the apex of the triangle representing P_2O_5=100 percent than the others, indicating a low K_2O and a high P_2O_5 in the NPK unit.

The relative positions of the check plot (No. 1) and of the plot receiving manure + CaO (No. 22), the lowest and highest yielding plots, are interesting. The composition of the NPK units with respect to N is nearly identical in both; with respect to K_2O the difference is not very great. The great differences in the yields of these two treatments are associable with relatively large differences in the intensity of nutrition and in the composition of the NPK unit with respect to P_2O_5 which is nearly 50 percent higher in plot receiving manure (No. 22).

Table 4.—*Comparison of the mean intensities of nutrition, mean NPK-units, and yield of P (plot No. 3), NP (plot No. 5), and lime (plot No. 23), with nothing (plot No. 1) and N (plot No. 2)*

Class order	Plot No.	Treatment	Intensity of nutrition	Composition of the NPK units			Yield
				N	P_2O_5	K_2O	
				Percent	*Percent*	*Percent*	*Pounds*
3	3	P	3.97	77.84	13.64	8.52	371.2
	5	NP	4.13	78.84	13.92	7.24	376.7
4	1	Nothing	4.62	73.67	5.74	20.59	165.4
	2	N	4.67	77.62	5.77	16.61	265.6
2	23	Lime	4.11	81.90	9.79	8.29	452.2
1	22	Manure	5.88	73.38	8.23	18.39	770.8

The importance of P_2O_5 is shown by the P plot (No. 3) as compared with the check plot (No. 1). A reduction of P_2O_5 in the NPK unit from 13.64 to 5.737, or approximately 58 percent, brought about a reduction in yield of grain, regardless of the simultaneous increase of K_2O in the NPK unit from 8.52 to 20.595, or approximately 142 percent, which occurred principally at the expense of the P_2O_5, though to a lesser extent at the expense also of the N in the NPK unit.

[8] See footnote 2, p. 685.

(C) THE RECIPROCAL EFFECTS OF CALCIUM AND POTASSIUM

The absorption of an element does not depend only upon the quantity of that element in the fertilizer, but on its quantitative relations to the other elements, even if there is not an absolute deficiency of them. Conclusions cannot, therefore, be drawn with respect to the nutrition of a plant from experiments in which the quantity of a single fertilizer element applied to the soil is the variable.

The addition of calcium in the form of superphosphate or in the form of lime lowers the level of nutrition with respect to potassium, producing very low values for K_2O in the NPK units. The causes lie in the changes in the equilibrium between K and Ca in the medium (soil), which result in a lower concentration of potassium in the soil.[9] This condition is reflected by the leaf with great sensitivity. Hence, in passing from a plot in which calcium dominates over potassium to a plot in which potassium dominates over calcium, the leaf faithfully reflects this inversion.

The effect on yields depends upon the level to which K or Ca has been reduced in the leaf. The mean K_2O content of the leaf in the optimum treatment—manure+CaO (No. 23)—is 2.44 percent, and the addition of calcium to the fertilizers on other plots, whether as superphosphate or as lime without potash additions, has reduced the level of K_2O in the leaf considerably below this optimum level. This reduction is a causal factor contributing to the reduced yields.

Thus through a deficiency of the medium (soil) in a certain element, the plant reacts by increasing the absorption of another element—an indication of the great changes possible (plasticity) in the composition of the plant.

SUMMARY

The nutrition of *Zea mays* growing on long-continued fertility plots receiving calcium from two sources, lime and superphosphate, is compared with that of plants on (1) a check plot receiving no fertilizer and no lime, (2) an unlimed plot receiving nitrogen only without superphosphate or potash, and (3) unlimed plots receiving superphosphate and potash with and without nitrogen (NPK, PK).

The results are recorded by means of graphs showing (1) the percentages of N, P_2O_5, K_2O, CaO, and MgO (as ordinates) and dates of sampling (as abscissae), and (2) the equilibrium between N, P_2O_5, and K_2O and the intensities of nutrition at the moment of sampling.

The effects of calcium from the two sources, lime and superphosphate, were similar, resulting in increased absorption of native soil nitrogen and depressed absorption of native soil potassium as well as of added potassium, with concomitant increases in the absorption of calcium and magnesium.

Calcium from both sources reduced the intensity of nutrition with respect to nitrogen, phosphoric acid, and potash ($N+P_2O_5+K_2O$) and also lowered the K_2O in the NPK unit from 20 to 8 (in round numbers).

Liming resulted in an increase in the nitrogen of the NPK unit and in a relatively great increase in the P_2O_5 made at the expense, principally, of the potash.

[9] THOMAS WALTER. THE DISTRIBUTION AND CONDITION OF THE POTASSIUM IN A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN CYLINDERS. Jour. Agr. Research 53: 533-546. 1936.

The yields of grain from the different treatments are classed in relation to the intensities of nutrition and the composition of the mean NPK units.

The lowest yields of grain are associated not with treatments (check, dried blood) having the lowest intensity of nutrition, but with the lowest P_2O_5 in the NPK unit. A reduction of 58 percent in P_2O_5 in the NPK unit of the check as compared with P brought about a reduction in yield of grain not offset by the accompanying increase of 142 percent in K_2O in the NPK unit which occurred at the expense, principally, of P_2O_5.

MONTHLY VARIATION IN CAROTENE CONTENT OF TWO IMPORTANT RANGE GRASSES, SPOROBOLUS FLEXUOSUS AND BOUTELOUA ERIOPODA [1]

By W. E. WATKINS [2]

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INTRODUCTION

Much of the semiarid mesa land lying in the south-central part of New Mexico along the Rio Grande has an average annual precipitation of from 8½ to 10 inches. Approximately half of this precipitation usually occurs in July, August, and September, the primary growing season during which most of the range grass is produced. Two of the most important range grasses of this area are black grama and mesa dropseed grass. These two grasses were selected in 1936 for a study of their nutritive properties at different stages of maturity.

Black grama (*Bouteloua eriopoda* (Torr.) Torr.), one of the very palatable grasses for livestock, grows on the mesas, hills, and dry open ground of large areas of range land in Texas, New Mexico, Arizona, and southern Utah. The upright stems of this grass remain green for a distance of 4 to 6 inches from their bases throughout the winter. Mesa dropseed grass (*Sporobolus flexuosus* (Thurb.) Rydb.) is found on the mesas of western Texas, New Mexico, Arizona, southern Utah, and southern California. It grows to a height of 20 to 30 inches and is very palatable throughout the growing season.

MATERIALS AND METHODS

The two grasses, black grama and mesa dropseed, were collected on the 15th of each month from an enclosure on a ranch of the New Mexico College of Agriculture and Mechanic Arts, located approximately 22 miles north of Las Cruces. The rainfall data were obtained from a rain gage on the experimental plot. The first sample was collected February 15, 1937, and similar collections will be made monthly until the completion of the project, which was planned to cover a period of 5 years. The bunches of grass in the plot were staked out in series of 12. The first series was harvested only on January 15 of each year and represents grass in an ungrazed pasture. The sixth series was harvested June 15 and contained some mature grass that had grown since June of the year before, along with new grass of the current season. The October series contained only the current year's growth at its approximate maturity. The black grama was cut 2½ inches and the mesa dropseed 3 inches above the surface of the ground. The samples were placed in canvas bags, weighed, taken to the laboratory, and subjected to analysis within an hour or an hour and a quarter after cutting. Occasionally, when it was not possible to begin the analysis

[1] Received for publication September 6, 1938.

[2] Acknowledgment is due R. W. Caldwell, of the California Agricultural Experiment Station at Davis, Calif., for valuable assistance in connection with certain phases of the project and for criticizing the manuscript.

immediately, the samples were placed in a mechanical refrigerator at a temperature of −3.0 to +1.0° C. and analyzed the following morning.

All the samples were finely pulverized in a Wiley mill except those containing fresh green growth with a moisture content of 50 percent or over, which were ground in a mortar with fine sea sand. All the samples, regardless of the method of grinding, were analyzed without drying as soon as they could be transported to the laboratory and prepared.

The plants were analyzed for carotene by the method of Guilbert,[3] with modifications suggested by R. W. Caldwell. Seven months after the beginning of this project, Munsey [4] published the results of his referee work on carotene methods. From reports and comparisons by other workers it is believed that the method used in the present work gives results that very closely approximate those obtained with the Fraps-Guilbert method used in the referee work. A 92-percent methyl alcohol wash solution was used instead of the 90-percent solution specified by the Guilbert method. Briefly, the procedure consists in subjecting the pulverized sample to saponification in aldehyde-free alcoholic potassium hydroxide and extraction with peroxide-free ethyl ether. The ether solution, after washing free from chlorophyllins, flavones, and alkali, is distilled under reduced pressure, the residue taken up in petroleum ether (boiling point, 35°–60° C.), and the xanthophyll removed by washing with 92-percent methanol. The carotene in petroleum ether solution, after making up to convenient volume, is determined colorimetrically, a dye solution prepared from orange G and naphthol yellow crystals being used for comparison. The dye solution was standardized against a sample of β-carotene [5] having a melting point of 184° C. Duplicate determinations were made upon the two range grasses and very close checks were obtained. The error averaged less than 4 percent.

ANALYTICAL RESULTS

The results of the analyses, presented in table 1 and figure 1, show that in 1937 the carotene value for black grama increased above the February and March levels to a high level in June, and then fell somewhat during July and August. This July and August period was rather dry and the grass made little growth, which may account for its low carotene content. The September value, which is the highest of the year, can be attributed to the fresh green growth that followed heavy early September rains. This growth continued at least until the middle of October, when the carotene value was approximately 15 percent less. The carotene values declined during the early winter months to a level of approximately 15 mg. per kilogram during February and March 1938. The black grama grass during February and March 1938 contained approximately three times the carotene content present during the same months of 1937. The winter of 1937–38, so far as minimum temperatures are concerned, was very mild. According to the records of the United States Weather Bureau station at State College, N. Mex., the average minimum monthly temperature for October 1936 and the three following months was

[3] GUILBERT, H. R. DETERMINATION OF CAROTENE AS A MEANS OF ESTIMATING THE VITAMIN A VALUE OF FORAGE. Indus. and Engin. Chem., Analyt. Ed. 6: 452–454. 1934.

[4] MUNSEY, V. E. REPORT ON CAROTENE. Jour. Assoc. Off. Agr. Chem. 20: 457–458. 1937.

[5] This sample was supplied through the courtesy of Dr. H. H. Strain, of the Carnegie Institution of Washington.

42.6°, 32.2°, 27.9°, and 21.4° F., respectively; the minimum monthly temperatures from October 1, 1937, to April 1, 1938, averaged 2.13° F. warmer than for the same period of 1936 and 1937. It is believed that this difference in temperature permitted a larger fraction than usual of the upright stems to remain green during the winter and thus furnished plant material with a higher carotene content.

Guilbert and his coworkers [6] found the minimum carotene requirement for cattle, sheep, and hogs to be 25 to 30 micrograms daily per kilogram of body weight. On this basis, the black grama grass contained, at its lowest level during February and March 1937, over three times the minimum amount of carotene needed for 900-pound range cattle during normal consumption of the dry grass. The carotene content of this grass seems to be influenced by stage of maturity, temperature, precipitation, and many other factors, but there nevertheless appears to be an abundance of carotene, even through the winter months, to satisfy the vitamin A requirement of range cattle.

TABLE 1.—*Monthly carotene content of black grama and mesa dropseed grasses and related meteorological data*

[Milligrams of carotene per kilogram of feed, dry basis]

Date	Carotene [1] content of—		Average minimum monthly temperature	Total precipitation	Date	Carotene [1] content of—		Average minimum monthly temperature	Total precipitation
	Black grama	Mesa dropseed				Black grama	Mesa dropseed		
1937	*Milligrams*	*Milligrams*	*° F.*	*Inches*	*1937*	*Milligrams*	*Milligrams*	*° F.*	*Inches*
Feb. 15	5.4	0	28.9	0.65	Nov. 15	80.0	0	30.5	-------
Mar. 15	5.8	0	33.4	.44	Dec. 15	26.6	0	29.0	0.48
Apr. 15	20.7	24.3	40.1	-------	*1938*				
May 15	32.7	37.5	50.1	.87					
June 15	82.8	81.7	57.9	.67					
July 15 [2]	52.5	93.7	65.1	.36	Jan. 15	24.1	0	27.0	.55
Aug. 15	38.2	84.7	65.5	1.04	Feb. 15	15.7	0	31.4	.39
Sept. 15 [2]	125.7	138.6	59.7	3.21	Mar. 15	13.4	0	35.1	.06
Oct. 15 [2]	105.9	68.4	46.2	.89	Apr. 15	[3] 26.5	0	40.5	.03

[1] Carotene results are averages of duplicate determinations.
[2] Average for April includes only 1 determination on black grama.
[3] Samples were ground in a mortar with sea sand.

The mesa dropseed grass during the growing season contained as much carotene, and in most cases a good deal more, than the black grama grass. However, the mesa dropseed grass dies with the coming of frost and freezing weather and the carotene is soon destroyed. In 1937 all the carotene was destroyed as early as November 15. This early loss of carotene suggests a somewhat limited winter usefulness of this grass except as a dry winter forage.

The seasonal characteristics of the two range grasses are clearly shown in figure 1. Although the amount of carotene in the black grama grass was slightly smaller than that in the dropseed grass during the growing season, the black grama was slower in maturing and maintained high carotene values for 30 days longer. The black grama always contained some carotene, even during the winter, when the amount was small, but no carotene was found in the dropseed from

[6] GUILBERT, H. R. See footnote 3.
——— MILLER, R. F., and HUGHES, E. H. THE MINIMUM VITAMIN A AND CAROTENE REQUIREMENTS OF CATTLE, SHEEP, AND SWINE. Jour. Nutrition 13: 543–564. 1937.

November 15 to April 15. The high carotene period of both grasses occurred during the early part of the growing season, which was influenced to a large extent by the exact time of the summer rains.

Other workers also have found a rapid change in carotene content of grass with change in season. Atkeson, Peterson, and Aldous,[7] of the Kansas Agricultural Experiment Station, reported that typical pasture plants showed relatively high carotene values in early summer, marked decreases during the hot summer months, and high carotene

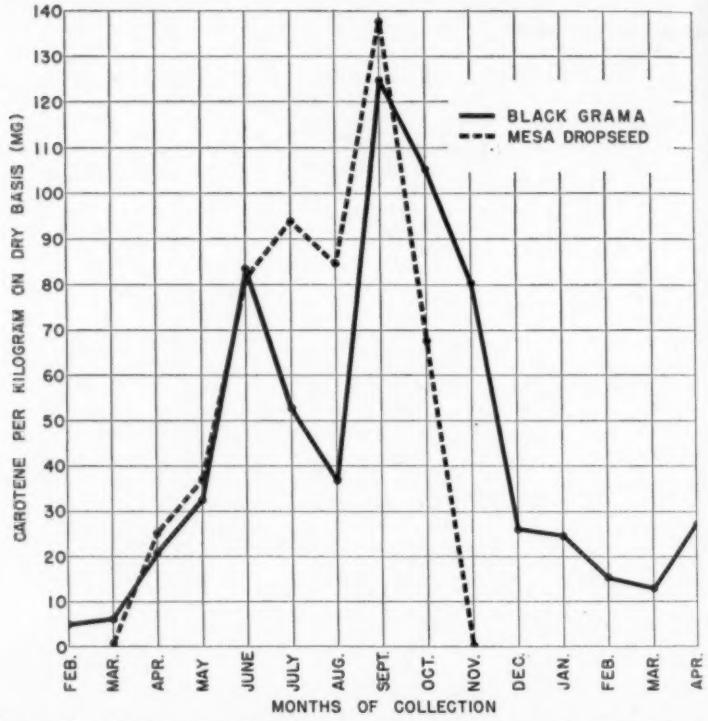

FIGURE 1.—Carotene content of two range grasses when samples were collected the 15th of each month.

values after the fall rains. Some of the native pasture grasses, such as big bluestem and buffalo grass, were very low in carotene content by November. Smith and Stanley,[8] of the Arizona Agricultural Experiment Station, working on the vitamin A potency of blue grama grass by the rat-growth method, found the early cut grass twice as potent as the mature grass and 100 times as potent as the November samples.

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[8] SMITH, MARGARET CAMMACK, and STANLEY, E. B. THE VITAMIN A VALUE OF BLUE GRAMA RANGE GRASS AT DIFFERENT STAGES OF GROWTH. Jour. Agr. Research 56: 69–71. 1938.

SUMMARY

The monthly carotene content of two important southern New Mexico range grasses, black grama and mesa dropseed, has been presented. Both grasses are moderately high in carotene during the growing season. The mesa dropseed loses all of its carotene soon after the fall freezes end the growing season. The black grama grass, whose upright stems remain green for a distance of from 4 to 6 inches of their base throughout the winter, contain an amount of carotene that seems to be ample to satisfy the vitamin A requirements of range cattle.

THE RELATION OF MOISTURE AND TEMPERATURE TO GROWTH OF THE COTTON ROOT ROT FUNGUS[1]

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INTRODUCTION

It is a matter of general observation by farmers and research workers that the spread of the cotton root rot fungus (*Phymatotrichum omnivorum* (Shear) Duggar) on cotton and other susceptible crops, and their resulting death, varies during the growing season to a rather large extent with available soil moisture. The disease spreads rapidly in hot weather following sufficient rainfall—that is, rains of about an inch or more. Taubenhaus and Dana (*8*)[2] analyzed air-temperature, rainfall, and air-humidity data as correlated with the percent of cotton plants killed by root rot during the years 1923, 1924, 1925, and 1926. They concluded that of the three factors, humidity has no direct influence; rainfall is outstanding in its importance; temperature for the crop-producing portion of the season is favorable to the development of the disease, and toward the end of the growing season lowered temperatures reduce root rot in severity in spite of favorable moisture. Taubenhaus and Ezekiel (*9*) reported a slow spread of the fungus from root to root throughout the winter to February 20 in two different fields near College Station, Tex.

To obtain a more direct relationship of moisture and temperature, especially the latter, to growth of the root-rot fungus, experiments were conducted under controlled conditions during the winters of 1931–32, 1932–33, and 1933–34. This paper gives the results of these studies.

METHODS

MOISTURE STUDIES

To determine the effect of moisture on the growth of the fungus and production of sclerotia, half-gallon size fruit jars were filled with soil of varying moisture content and inoculated with pieces of freshly infected cotton roots. The surface soil of Houston black clay was air-dried in the greenhouse for a few days, being turned at intervals to secure uniform drying. The moisture content of the air-dried soil was usually around 8 percent, oven-dry basis. In these experiments the cultures were set up so that soil moistures of 15, 20, 25, 30, 35, 40, and 45 percent, on the oven-dry basis, were obtained. These are equivalent to 21, 28, 35, 42, 49, 55, and 62 percent, respectively, of the maximum water-holding capacity as determined by the Hilgard method. Since water was mixed in an open container with the soil, a small amount was added in excess to take care of evaporation during the mixing process and while the soil was being transferred to jars.

[1] Received for publication September 17, 1938. Contribution No. 477, Technical Series, Texas Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 708.

Samples were taken from each batch of soil to check the moisture content. All soil was rubbed through a quarter-inch mesh sieve while it was being mixed. After half a jar was filled with soil, five pieces of infected root inoculum were stuck vertically in the jar, and the remainder of the soil was added. In the case of higher moisture percentages, the soil moisture was made to about 25 percent by hand, and the remainder of the water added directly to the jar containing the soil.

Fungus growth was measured by methods described under Growth Measurements.

TEMPERATURE STUDIES

For making the temperature studies a water bath was constructed so that a series of nine different temperatures could be obtained at the same time. Sectional views of the apparatus are shown in figure 1. This equipment was constructed by making a single box of 20-gage galvanized iron, 126 inches long, and adding partitions of the same material so that nine compartments were formed, each 14 by 14 inches.

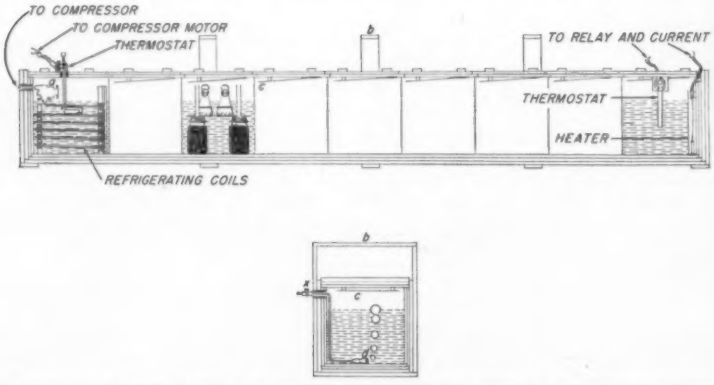

FIGURE 1.—Sectional views of water-bath apparatus used in making temperature studies.

Around this two layers of ½-inch Celotex were added and boxed in with 1-inch thick shiplap. Small slats were nailed on the bottom, around the sides, and across the top to support the box off the floor and to hold the shiplap against the Celotex (fig. 1, *b*). Covers for each compartment were made of two pieces of ½-inch Celotex held together by small slats. A piece of sheet iron was fastened at an angle on the under side of each cover so that water of condensation drained off to one side (fig. 1, *c*). Refrigerating coils were placed in one end of the box and connected with an ordinary household refrigerant compressor and motor assembly. Beginning with the expansion valve (fig. 1, *a*) coiling was begun at the top so that the coldest part of the coils was at the top of the water bath. Refrigeration was controlled by a bimetallic thermostat. In the opposite end high temperatures were obtained by housing the element from an electric oven in a copper box and connecting with the current through a relay of the type used on electric ovens and a pencil-type bimetallic ther-

mostat. In this manner a series of different temperatures resulted across the nine compartments that varied with the change of the two thermostats. Agitation was obtained in each water bath by compressed air. An air line, made of copper tubing connected with an automatic air compressor, provided air for each compartment through an inlet of the same size tubing connected with heavy-walled rubber hose and controlled by a pinch clamp (fig. 1, *x*). A small boxlike piece of sheet iron (fig. 1, *d*) was soldered to the end of each inlet at the bottom of its respective compartment and tilted at a slight angle downward. In this way a large amount of air accumulated in the small box before a bubble escaped. As a result of this, the bubbles were large and adjustments were made so that they escaped at the rate of about one each 5 seconds. Pressure in the air line was kept at 15 pounds by a regulator to which a filter and gage were attached. During the course of each experiment the entire apparatus, shaded with opaque paper, was housed in the greenhouse thermostatically controlled at 65° F. The temperature in each compartment seldom varied more than 1° C. The apparatus as set up in the greenhouse is shown in figure 2.

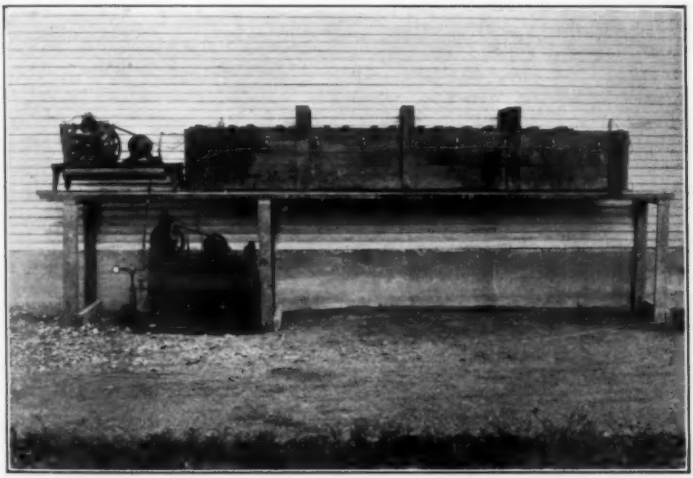

FIGURE 2.—Apparatus used for making temperature studies as set up in the greenhouse.

Growth responses were obtained in soil cultures in jars and in pure cultures on liquid media at the same time. Holes were punctured in the jar lids, and a 6-inch tube was fastened thereto so that it extended above the water line. A piece of hardware cloth was set on top of the jars, and the pure cultures in Erlenmeyer flasks were set on top of the hardware cloth. Each flask was weighted down with a lead ring. The jar cultures were made similar to those described under the moisture tests, and dry weights were obtained from liquid cultures made by using a medium constituted as follows: Water 1,000 cc, ammonium nitrate 1.2 g, potassium phosphate (secondary) 1.4 g, magnesium sul-

phate 0.8 g, potassium chloride 0.15 g, sucrose 30g, starch 30 g. Fifty cubic centimeters of this medium, which was made to pH 6.8, was used per flask and inoculated with small cubes of agar about 2 mm thick, containing young mycelial growth. Five jars and six flasks were used in each compartment for each experiment. At times *Rhizopus* or other fungi grew through and produced spores on the bottom of the cotton plugs. Such contamination was prevented by flaming the plugs and covering with sterilized sheet dental rubber.

GROWTH MEASUREMENTS

Whether *Phymatotrichum omnivorum* is growing superficially on culture media or along roots, compound mycelial strands are formed over the entire surface. These structures have been described previously (*1*, *2*, *6*). Toward maturity they assume a buff or brown color and may extend through the soil as much as 8 or 10 inches in either direction. The number and length of these strands as shown on the walls of the glass jars was used as one measurement of growth response. This applied only to the soil cultures in both moisture and temperature studies. Sclerotia are formed at intervals in strands by the compounding and compacting of hyphae. They are of great importance in making studies of cotton root rot because of their ability to remain in a viable state for many years and the fact that they may be formed in the soil at depths up to 4 feet or more. The number of sclerotia produced was used as another measurement of growth response in the soil-moisture and temperature cultures. Sclerotia were separated from the soil by methods previously described (*3*). In the pure cultures on liquid media growth was measured by obtaining dry weights of fungus, including the mat growing directly in and on the media and the strand mycelium and sclerotia growing on the flask walls.

RESULTS

SOIL-MOISTURE STUDIES

Production of strands and sclerotia in four different soil-moisture experiments are given in table 1. In the data the number and length of strands represent the average per jar in each individual test, set up in triplicate or quadruplicate for each different moisture percentage. These particular tests were conducted at different times during a 3-year period. The variation from test to test might therefore be caused by any one or all of three or four different factors, primarily differences in temperature from season to season and some differences in inoculum for the different years, or differences within the same year in age of plants, since in all cases cotton roots were used as the source of inoculum. Strands were produced in abundance in even the comparatively dry soil, diminishing rather sharply at and above 35-percent moisture content.

Sclerotia were produced somewhat sparingly even in those soils with optimum moisture for strand production. The optimum for both strand and sclerotial production seems to be from 25 to 30 percent. The number and length of strands is not always indicative of the growth in any culture. These strands branch rather freely in a dichotomous manner. Sometimes a single strand will cover two sides or more of a half-gallon jar. Over a number of cultures, however, the

number and length of strands is indicative of the growth response under any given set of conditions. Sclerotia and strands in the drier cultures had a downy covering of hyphae of the acicular type. In the soils having higher moisture percentages, especially those nearing soil saturation, the strands were smoother and did not show much of the acicular-hyphae covering.

TABLE 1.—*Production of strands and sclerotia in soil cultures with quantities of water varying from 8 to 40 percent* [1]

Duration of experiment (days)		15 percent	20 percent	25 percent	30 percent	35 percent
5	Strands, number	1.0	3.0	4.3	0.7	1.3
	Strand length, centimeters	.8	.3	1.0	.3	.7
	Sclerotia, number	.0	.0	.0	.0	.0
24	Strands, do	9.8	5.8	12.0	8.5	2.5
	Strand length, centimeters	6.4	4.1	6.3	4.8	1.6
	Sclerotia, number	11.0	4.0	2.0	.0	.0
72	Strands, do	12.3	10.3	7.3	2.3	.3
	Strand length, centimeters	8.3	7.3	8.8	14.3	.7
	Sclerotia, number	5.0	.0	10.0	91.0	.0
120	Strands, do	1.7	6.0	8.7	4.7	6.3
	Strand length, centimeters	1.4	5.4	6.2	6.1	9.8
	Sclerotia, number	.0	.0	5.0	.0	.0

[1] No growth at 8-percent, air-dry, nor at 40-percent moisture.

TEMPERATURE STUDIES

The average weights of mycelial growth per 250-cc flask from four different temperature experiments are shown in tables 2 and 3. In the last two experiments (table 3) the heat-control thermostat was changed from 50° to 45° C.

TABLE 2.—*Average dry weight of root-rot fungus per 250-cc flask after 10 and 20 days growth at different temperatures from 3° to 50° C.* [1]

Growth period (days)	Dry weight when grown at—			
	19°	24°	29°	34°
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
10	7	99	139	127
20	45	403	506	404

[1] No growth at 3°, 12°, 39°, 44°, or 50°C.

TABLE 3.—*Average dry weight of root-rot fungus per 250-cc flask after 10 and 60 days growth at different temperatures from 3° to 45° C.* [1]

Growth period (days)	Dry weight when grown at—					
	11°	18°	23°	27°	32°	37°
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
10	0	6	27	170	218	4
60	30	336	492	830	390	15

[1] No growth at 3°, 42°, or 45° C.

The optimum range for fungus growth is from approximately 23° to 34° C., the middle of this range being 27° to 29°, as shown by the experimental results. In the first experiment an average of 506 mg of fungus was obtained after a 20-day growth period at 29°. In the last experiment at the end of 60 days an average of 830 mg was obtained at 27°, these figures representing the maximum growth in each experiment. At all times in the 3° bath there was a rim of ice on the coils that almost reached the center of the compartment. Therefore the flasks in this compartment were surrounded by ice at least part of the time. Hyphae in the flasks in this coldest chamber grew about 2 mm during the first 2 days but stopped thereafter, and the tips seemed to curl backward in a somewhat hooked manner. Although there was no growth under such low-temperature conditions, the fungus readily resumed growth when transferred to warmer baths at 27° or 29° even at the end of the longest period of 60 days. At the higher temperatures the effect was directly opposite. At 39° or above no growth was obtained, and when cultures were transferred to 27° or 29° baths there was no resumption of growth, even at the end of the shortest experimental period of 10 days. This indicates that all fungus growth had been killed.

Microscopic examination of mycelial growth showed no general morphological differences. There was one striking difference in appearance. At the lower temperatures up to 19° C. the mycelium remained almost white, never getting darker than a light amber color. With increase in temperature the entire mycelium showed a darker color regardless of the amount of growth. The same thing held true in the case of the soil cultures at different temperatures. Both strands and sclerotia were formed with the light or whitish color that the mycelium usually exhibits in the younger stages of growth, retaining this light color throughout the experimental period. Throughout the optimum-growth range the fungus exhibited characteristics typical of those observed under field or ordinary laboratory conditions—that is, at first there was a whitish web which gradually increased in size and became darker until the brown or buff color of maturity was attained. At the higher temperatures, at and above 34°, the mycelium was first formed with the dark color of maturity and retained this or became darker throughout each growth period Color, therefore, is not necessarily an indicator of age of sclerotia, as is generally thought to be the case. In field areas almost all light-colored sclerotia are found in the late fall or early winter after cool weather has begun.

The number of sclerotia produced at different temperatures after 30-, 60-, and 80-day growth periods are shown in table 4. Each of these different periods represents a separate experiment, all the jars in each chamber being analyzed for sclerotia for each separate experiment. Where sclerotia are formed under such conditions they are usually produced within the first 30 days and certainly none after 80 days, because the inoculum or substratum has been depleted and decomposition has set in. Optimum temperature for sclerotial formation is somewhat the same as that for general mycelial growth, although in the 80-day experiment the greatest abundance of sclerotia was produced at 18° C. The greatest amount of strand formation and sclerotial production was obtained at 27°.

TABLE 4.—*Production of root-rot sclerotia in soil cultures at different temperatures from 3° to 45°C. after 30, 60, and 80 days* [1]

Growth period (days)	Number of sclerotia when grown at—					
	11°	18°	23°	27°	32°	37°
30	0.0	0.0	95.0	207.0	29.0	0.0
60	.0	.0	.0	209.0	8.0	52.0
80	66.2	293.8	131.6	147.2	12.0	8.8

[1] No growth at 3°, 42°, or 45° C.

DISCUSSION

As pointed out by Taubenhaus and Dana (*8*) and as commonly observed, moisture is the main limiting factor for root-rot spread during the growing season. The temperature is sufficiently high from May through October for ready growth of *Phymatotrichum omnivorum*. Usually by the first of May the ground is sufficiently warm for rapid spread of the fungus, if host material is present in a fairly dense stand. After rains in July, August, and September there is usually a rapid spread of root rot in cottonfields as evidenced by the wilting and speedy death of the cotton plants. In years of low soil moisture throughout the growing season root rot is held in check. In such years there may be a general kill of 25 percent, whereas in years of intermittent rainfall throughout the growing season the amount of cotton killed may be as high as 50 percent. Early or midseason killing of cotton plants results in low yields (*5*).

These moisture and temperature experiments and general field observations indicate that *Phymatotrichum omnivorum* thrives over a rather wide range of moisture and temperature conditions. The fungus is, however, readily affected by high temperatures and low moisture conditions. This accounts for the fact that no mycelial strands and sclerotia are found in the upper 6 inches of soil except in periods of warm wet weather. At such times the white mycelium may be found or seen growing as a collar around the stalks of cotton or other host plant just at the ground line. It is also during these periods that the mycelium grows to the surface and produces the downy conidial mats which may vary from the size of a dime to 18 inches or more in diameter. These structures are formed in the shade of low-growing field crops or sometimes under bushes or trees where the soil is not subject to the drying effect of the sun's rays and remains moist for a few days. Sclerotia have been found thus far to remain in a viable condition for 8 years in the soil (*4, p. 204; 7*). If these bodies are left exposed in the open, they lose their viability within a very short period, usually less than an hour, depending on air temperature and humidity. There is a decided difference in this respect between the sclerotia of *Phymatotrichum omnivorum* and those produced by certain other fungi. The mycelial strands also rapidly lose their viability under the same conditions.

Taubenhaus and Ezekiel (*9*) found that there was a spread of root rot along the cotton roots throughout the winter near College Station, Tex. This district is near the lower end of the blackland section, the section where cotton root rot is worst, but it is quite probable that

such spread occurs in all parts of the blackland. Soil temperature records taken at the Temple Substation at depths of from 2 to 48 inches show that at the 18-inch depth or deeper the temperatures are never too cold for growth of *Phymatotrichum omnivorum*. There is no doubt, however, that growth is slowed down greatly during the winter. Host material is the main limiting factor in the spread of the fungus. Active root rot has not been found earlier than April on winter annual weeds. The growth reported by Taubenhaus and Ezekiel was on old cotton plants growing in the field from the previous season. It is necessary that cotton plants become fairly well established before attacks by the fungus occur, since no seedlings have been found dying from the cotton root rot disease. This is partially owing to the fact that there is little or no mycelial growth in the upper layers of the soil.

SUMMARY

Mycelial strands of the cotton root rot fungus (*Phymatotrichum omnivorum* (Shear) Duggar) grew in Houston black clay soil in which the soil moisture varied from 15 to 35 percent, oven-dry basis. The optimum moisture content was around 25 percent, which is equivalent to 35 percent of the maximum water-holding capacity of the soil. Sclerotia of *P. omnivorum* were produced at a soil moisture content of from 15 to 30 percent. Neither sclerotia nor mycelial strands grew at or below 8 percent or above 35 percent soil moisture.

Both mycelial strands and sclerotia were produced in constant-temperature water baths at temperatures of from 11° to 37° C., the optimum for both being approximately 27°.

At the lowest experimental temperature of 3°, there was no active growth, but apparently the fungus was not injured. Temperatures of 39° and above resulted in the death of the fungus.

At the lower temperatures both the sclerotia and mycelium formed were of a whitish-amber color. At the minimum temperature at which growth occurred this light color was retained during the entire experimental period.

At higher temperatures both sclerotia and mycelium were formed with the usual dark color of maturity, as found in specimens taken from field soil.

Both mycelial strands and sclerotia were rapidly killed by exposure to high temperatures or to drying, such as takes place under ordinary field conditions in the summer.

Temperatures throughout the crop-growing season are favorable for growth and spread of *Phymatotrichum omnivorum*. During this period, moisture is the limiting factor, intermittent rains of sufficient quantities causing rapid spread of the fungus, a large proportion of the cotton plants being killed by the end of the season.

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BILATERAL VARIATION IN THE WEIGHT AND COMPOSITION OF THE LONG BONES OF SOME EXPERIMENTAL ANIMALS [1]

By CHAS. E. WEAKLEY, Jr., *associate chemist*, and R. B. DUSTMAN, *head, Department of Agricultural Chemistry, West Virginia Agricultural Experiment Station*

INTRODUCTION

The literature dealing with bilateral variation in the bones of experimental animals and suitable for close comparisons is meager and scattered. Investigators have sometimes used bones from one side of an animal for one type of determination and the corresponding bones from the other side for a different type of determination, with the tacit assumption that the bones from the two sides were identical in composition or other properties. Some years ago an investigation was begun at this laboratory to determine the composition of various skeletal parts of experimental animals. This afforded an opportunity for a study of bilateral variation in the long bones.

MATERIALS AND METHODS

In the study herein reported four kinds of animals were under experiment, viz, dairy cattle, swine, chickens, and rats. In all cases the animals were on feeding trials designed to produce differences in bone development; hence the data include bilateral comparisons in individuals on both normal and deficient rations. In order to obtain as accurate a representation as possible of the bone in its natural state and to avoid variations in sampling, whole green bones were used for analysis throughout. In certain instances two or more bones were combined to form a single composite sample. The bones taken for study from each type of animal were as follows:

Rat: Femur, tibia, and fibula (composite); humerus, ulna, and radius (composite). Total of four samples.

Chicken: Femur; humerus; tibia; ulna and radius (composite). Total of eight samples.

Swine: Femur; humerus; tibia; ulna and radius (composite); seventh and eighth ribs (composite). Total of 10 samples.

Dairy cow: Femur; humerus; tibia; metacarpus; metatarsus; ulna and radius (composite); seventh and eighth ribs (composite); thirteenth rib. Total of 16 samples.

The bones were removed from the fresh carcass, freed from adhering tissue, and weighed. In dissecting out the bones all muscle, tendons, and ligaments were carefully removed. The periosteum and articular cartilages, however, were left intact on the shaft and so were included in all samples.

The swine and cattle bones were ground first in a power-driven Mann bone grinder and immediately thereafter in a small hand-operated food chopper. The few pieces failing to pass through the grinding machine were carefully collected and reduced by hand in a

[1] Received for publication September 26, 1938.

covered iron mortar. After drying in vacuo and extracting in large Soxhlet extractors, the resulting moisture-free, fat-free material was ground finely in a Wylie mill and the ash determined.

The chick and rat bones were crushed between the jaws of pliers, cut into small lengths with diagonal cutting pliers, dried in vacuo, extracted in a modified Bailey-Walker extraction apparatus,[2] and ashed in fritted-bottom Jena glass crucibles without transfer after preparation for drying.

The determinations made on all bones were green weight, moisture, ether extract, ash, and organic nonfat (by difference).

Since the determinations made on each animal were paired throughout as lefts and rights, the differences found were studied statistically by Student's method and compared according to Livermore's table of odds [3] as shown in table 2.

The 20 rats used were albinos—10 males and 10 females; 5 of each sex were on a normal ration and 5 on a rachitic ration. The rats were killed at 65 and 70 days of age.

The 20 chicks used were White Leghorns—10 males and 10 females; 5 of each sex were on a normal ration and 5 on a rachitic ration. The bones were taken at 35 days of age.

The 19 hogs included 8 Poland China, 4 Duroc Jersey, 3 Berkshire, and 4 cross-bred animals. There were 14 males and 5 females. The animals ranged in age from 193 to 288 days at time of slaughter.

Two grade Holstein heifers supplied the bones for the comparisons with dairy cattle. One was fed a normal ration and the other was fed a ration low in phosphorus. After dissection from the carcass, all bones were broken open with a cleaver and the marrows were separated and removed as completely as was practicable from all except the ribs. Marrows and bones exclusive of marrows were weighed and analyzed separately. The analytical results were combined later to represent the values for whole bones. The animals were 15 months old at time of slaughter.

EXPERIMENTAL RESULTS

The average values for the various bones and determinations are shown in table 1. An examination of this table reveals some slight difference between the left and right fore and rear composites in the rat, but a statistical examination of these differences shows them to be without significance for 9 of the 10 comparisons made, the exception being ether extract in the tibia-fibula-femur composite sample. In 1931 Jones and Robson [4] reported separately the ash content of right and left femurs from 32 adult rats. From their data the average percentage of ash may be calculated as 62.89 for the right and 62.90 for the left femur, respectively, in the dry, extracted bone. Hume, Pickersgill, and Gaffikin [5] also reported the ash content of right and

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left composite samples of tibia and femur for 88 individual rats, and a calculation of the average percentage of ash in dry extracted bone from their data yields identical values of 44.28 for either right or left. In the present investigation the ulna and radius were combined with the humerus, and the tibia and fibula were combined with the femur on either side. The bones from the left foreleg average 0.3 percent higher in ash (green basis) than those from the right foreleg, but the bones from the right rear leg are higher than those from the left by a like amount. It seems, therefore, that insofar as comparisons can be made, these data are in agreement with those of other investigators in showing that in the rat there is no significant difference between the ash content of long bones from the right and left sides. Nor is there any significant difference in weight of green bone, percentage of moisture, or organic nonfat in the bones from the two sides. In the rear leg the ether extract is significantly higher in the bones from the left side, but in the foreleg the trend is toward higher values on the right side. When the values for fore and rear legs are combined the difference between left and right is without significance.

TABLE 1.—*Average weight and composition of rat, chicken, swine, and dairy-heifer bones on green basis*

RAT

Bone	Green weight		Moisture		Ether extract		Ash		Organic nonfat	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
	Grams	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ulna, radius, and humerus	0.2541	0.2543	47.03	47.07	2.05	2.17	25.77	25.47	25.15	25.29
Tibia, fibula, and femur	.6134	.6093	50.15	49.91	3.91	3.77	22.27	22.58	23.67	23.74

CHICKEN

Ulna, radius	0.7016	0.6934	56.73	56.04	7.64	7.86	14.70	14.91	20.93	21.19
Humerus	.9718	.9340	61.99	61.19	4.23	4.00	13.17	13.89	20.61	20.92
Femur	1.2021	1.2119	60.79	60.54	4.30	4.44	13.95	14.12	20.96	20.90
Tibia	1.5594	1.5166	60.02	59.62	5.84	5.83	14.43	14.68	19.71	19.87

SWINE

Ulna, radius	182.94	181.80	26.27	26.17	15.02	15.33	36.37	36.28	22.34	22.22
Humerus	254.43	252.40	27.76	27.58	18.59	18.93	33.29	33.39	20.36	20.10
Femur	276.12	274.35	27.90	27.53	20.43	20.49	32.13	32.44	19.55	19.54
Tibia	171.21	169.77	25.46	24.93	17.51	17.75	35.70	36.06	21.34	21.27
Ribs, seventh and eighth	67.64	66.83	30.59	30.54	10.63	10.42	35.72	36.12	23.07	22.92

DAIRY HEIFER

Metacarpus	280.5	291.5	24.18	25.82	24.65	23.24	31.12	30.75	20.05	20.19
Ulna, radius	657.8	673.7	27.61	27.61	26.50	26.83	26.71	26.51	19.18	19.05
Humerus	858.9	862.2	33.44	33.27	26.75	27.53	21.83	21.75	17.98	17.45
Femur	1,196.2	1,206.0	34.60	36.50	28.17	25.38	20.63	20.65	16.60	17.47
Tibia	765.6	790.7	28.20	29.62	26.57	23.94	26.87	27.63	18.36	18.81
Metatarsus	342.0	351.2	21.21	21.95	26.73	25.18	32.05	32.10	20.01	20.77
Ribs, seventh and eighth	286.8	294.5	46.89	43.83	7.51	10.01	23.34	23.81	22.26	22.35
Rib, thirteenth	51.3	51.1	40.39	38.56	4.98	5.67	29.26	29.92	25.37	25.85

Turning next to a consideration of the data from chick bones (table 1), it is apparent that a somewhat different situation exists. For all bones analyzed, except the femur, the average weight is greater on the left side, as is percentage of moisture in all instances, while percentage of ash is greater on the right side. Apparently green weight and moisture content tend to run higher on the left side, whereas ash varies inversely with moisture and is higher for the bones of the right side. Table 2 shows the significance of the differences between the two sides for individual bones and for combined values. For combined values the odds are significant for all determinations made on chick bones except ether extract.

TABLE 2.—*Approximate odds that bones from one side exceed those from the other side with respect to a given determination*

Animal and bone	Analyses on each side	Green weight	Moisture	Ether extract	Ash	Organic nonfat
Rat:						
Ulna, radius, humerus	*Number* 20	*Grams* R>L 1:1	*Percent* R>L 1:1	*Percent* R>L 5:1	*Percent* L>R 4:1	*Percent* R>L 3:1
Tibia, fibula, femur	20	L>R 3:1	L>R 5:1	L>R 55:1	R>L 9:1	R>L 3:1
Chick:						
Ulna, radius	20	L>R 4:1	L>R 325:1	R>L 16:1	R>L 6:1	R>L 32:1
Humerus	20	L>R 500:1	L>R 269:1	L>R 2:1	R>L 3, 200:1	R>L 21:1
Femur	20	R>L 4:1	L>R 18:1	R>L 7:1	R>L 14:1	L>R 3:1
Tibia	20	L>R 240:1	L>R 18:1	L>R 1:1	R>L 12:1	R>L 17:1
Swine:						
Ulna, radius	19	L>R 7:1	L>R 3:1	R>L 15:1	L>R 2:1	L>R 11:1
Humerus	19	L>R 32:1	L>R 7:1	R>L 18:1	R>L 3:1	L>R 26:1
Femur	19	L>R 11:1	L>R 49:1	R>L 2:1	R>L 2:1	L>R 1:1
Tibia	19	L>R 15:1	L>R 624:1	R>L 4:1	R>L 22:1	L>R 3:1
Ribs, seventh and eighth	19	L>R 9:1	L>R 2:1	L>R 8:1	R>L 26:1	L>R 26:1
ombined values for all bones of—						
Rat [1]	40	L>R 2:1	L>R 2:1	L>R 1:1	R>L 1:1	R>L 4:1
Chick	80	L>R 500:1	L>R 10,000:1	R>L 4:1	R>L 3,000:1	R>L 115:1
Swine	95	L>R 1,000:1	L>R 1,000:1	R>L 14:1	R>L 300:1	L>R 250:1
Dairy heifer [2]	16	R>L 121:1	R>L 1:1	L>R 4:1	R>L 7:1	R>L 23:1

[1] If weights from an additional series of 19 rats killed 2 years prior to those given in this table are included, the total number of bones weighed from each side becomes 78 and the odds for weight become 3:1 that L>R.
[2] Since bones were analyzed from only 2 dairy animals the odds for individual bones are omitted.

The swine bones (table 1) show relationships in general agreement with those set forth for the chick bones. Without exception the average values for green weight and moisture content are higher on the left side than on the right, while in all instances but one the percentage of ash is higher in the bones of the right side. Table 2 shows that here, also, the odds for combined values are significant for all determinations except ether extract. The direct relation between green weight and moisture content and the inverse relation between moisture and ash content may be shown by constructing simple charts or scatter diagrams (not included herewith) setting forth the individual instances in which both determinations run higher on the same side and the corresponding instances in which the higher values are found on opposite sides. If this is done for the 95 pairs of hog bones it will be found that in 62 of the comparisons the percentage of moisture varies directly with green weight, while in 71 of the comparisons the higher percentage of ash is on the side opposite the higher percentage of moisture. The charts will show further that no single

bone or group of bones is responsible for this relationship or for the dominance of one side over the other, but that all bones share in the relationship. Breed and sex may likewise be shown to be without pronounced effect.

If we designate as 5–L an animal in which all five bones on the left side are heavier than the corresponding bones on the right side, as 4–L an animal in which all four bones on the left side are heaver than the corresponding bones on the right, etc., a table may be constructed showing the relationships of breed to dominance in weight. Table 3 gives such data.

TABLE 3.—*Tendency by breeds of swine toward dominance of one side over the other side in weight of green bone*

Breed	Individuals in each group [1]					
	5-L	4-L	3-L	3-R	4-R	5-R
	Number	*Number*	*Number*	*Number*		
Poland China	1	2	3	2	None	None
Duroc Jersey	1	2	0	1	...do...	Do.
Berkshire	0	1	0	2	...do...	Do.
Cross-bred	0	1	2	1	...do...	Do.
Total	2	6	5	6	0	0

[1] 5–L indicates an animal with all 5 bones on the left side heavier than the corresponding bones on the right side, 4–L one with 4 and 3–L one with 3 bones on the left side heavier than the corresponding bones on the right side. 3–R indicates an animal with 3 bones on the right side heavier than those on the left.

From these figures it appears that in the 19 hogs studied there were no strongly right-dominant individuals, but there were a number of strongly left-dominant ones. The data indicate further that no single breed is chargeable with the tendency toward heavier bones on the left side, but that all seem to contribute to this end. On the other hand, at least one individual of each breed and one cross-bred animal showed moderate right dominance.

The number of dairy animals available for study was too small to be of any considerable value in establishing clear-cut tendencies in bilateral comparisons. However, the average values presented in table 1 for the two heifers studied show an unmistakable trend toward heavier bones on the right side and a direct relation of moisture to green weight in 12 of the 16 comparisons, while the inverse relation of ash to moisture holds for 11 of the comparisons as shown by scatter diagrams (not included herewith). The odds for right or left dominance in moisture and ash are not significant, but odds for right dominance in organic nonfat approach significance.

The organic nonfat fractions of the chick and hog bones afford an interesting and somewhat puzzling comparison. The combined values in table 2 show that in swine organic nonfat is significantly higher on the left side along with green weight and percentage of moisture. In the chick, on the other hand, organic nonfat is significantly higher on the right side although green weight and moisture are higher on the left side as in the hog bones.

A study of comparative weights (rather than percentage) of moisture, ether extract, ash, and organic nonfat in the corresponding

left and right bones from chicks and swine shows that where an appreciable difference in total weight of green bone exists between the two sides, this difference is likely to be chiefly in the moisture fraction, although the other constituents also may contribute to the difference in total weight.

SUMMARY AND CONCLUSIONS

In a study of bilateral variation in the weight and composition of green bones of 20 rats, 20 chicks, 19 hogs, and 2 dairy heifers the following results were obtained:

(1) No significant differences in weight of green bone or in percentage of moisture, ash, and organic nonfat were found in bones from the right and left sides of the rat.

(2) In the chick bones green weight and percentage of moisture were significantly higher on the left side while percentage of ash and organic nonfat were significantly higher on the right side.

(3) In the hog bones green weight, percentage of moisture, and percentage of organic nonfat were significantly higher on the left side while percentage of ash was significantly higher on the right side.

(4) The dairy heifers showed a tendency toward heavier bones on the right side.

(5) In animals in which one side showed a tendency to dominate in weight of bone the same side tended to be higher in percentage of moisture and the opposite side to be higher in percentage of ash.

These results may be interpreted as suggesting (1) that certain species of lower animals may tend toward a dominance of one side over the other with respect to bone development, and (2) that bones from one side are not necessarily identical in composition or other properties with those from the other side, although in general the differences found in these experiments were quite small.

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